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NOTEWORTHY COLLECTION

CALIFORNIA

CEPHALANTHERA AUSTINIAE (A. Gray) A. Heller (ORCHIDACEAE). — Santa Barbara County, Santa Ynez Mountains, Kinevan Canyon, 34.50497 N, 119.817505 W, 580 m (1900 ft) elevation, south side of Kinevan Road, above San Jose Creek; ca. 0.2 km west of junction with West Camino Cielo, which is 0.3–0.4 km WNW of junction with State Hwy 154, 6 May 2015, *Steven Timbrook s.n.* (SBBG 129335). Stem and leaves white; flower white with yellow spot on lip. Within 1 m of pavement in shaded leaf litter over loam on north-facing slope in mixed forest of *Notholithocarpus densiflorus* (Hook. & Arn.) Manos et al., *Quercus agrifolia* Née, and *Umbellularia californica* (Hook. & Arn.) Nutt., with *Dryopteris arguta* (Kaulf.) Maxon, *Ribes amarum* McClatchie, and *Scrophularia californica* Cham. & Schltdl.

Two additional plants were observed along West Camino Cielo Road between the junction with Kinevan Canyon Road and bridge over San Jose Creek.

Previous knowledge. Cephalanthera austiniae is the only North American species of this largely Eurasian genus. It is a mycoheterotrophic orchid found in rich soils of mixed-evergreen or coniferous forests of southwestern British Columbia, Canada, Washington, Oregon, Idaho, and California. Herbarium specimens from California document its occurrence in the northwest, the San Francisco Bay region, the Cascade and Sierra Nevada ranges, the San Bernardino Mountains, and the outer South Coast Ranges in Riverside and San Diego counties.

Significance. This collection is the first from Santa Barbara County, the Santa Ynez Mountains, and the Transverse Ranges. The nearest previously known collections are from the Santa Lucia Mountains, Monterey County, approximately 225 km NNW, and Crestline, San Bernardino County, approximately 235 km ESE. *Cephalanthera austiniae* is not reported in the most recent flora of the Santa Barbara Region (Smith 1998). Kinevan Canyon, the site of this collection, has species including *Notholithocarpus densiflorus* and *Vaccinium ovatum* Pursh that are indicators of north coastal forests.
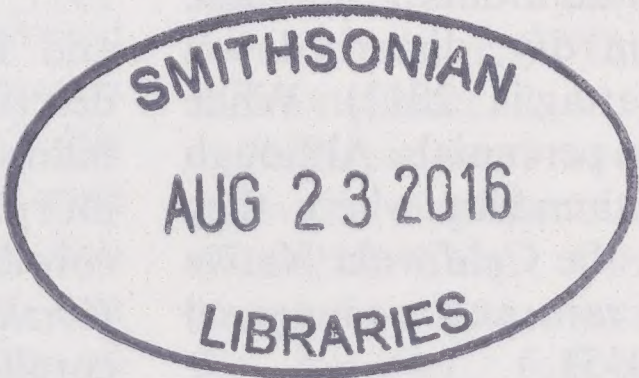
—STEVEN TIMBROOK, 2412 Foothill Road, Santa Barbara, CA 93105; stevetimbrook@cox.net.

LITERATURE CITED

SMITH, C. F. 1998. A flora of the Santa Barbara Region, California. Santa Barbara Botanic Garden & Capra Press, Santa Barbara, CA.

TOWARDS A COMPLETE SPECIES LEVEL PHYLOGENY OF *LEPTOSIPHON* (POLEMONIACEAE)

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ABSTRACT

Leptosiphon Benth. (Polemoniaceae) has 40 taxa, distributed mainly in western North America, with one species in Chile. Phylogenetic studies of Polemoniaceae have consistently supported recognition of *Leptosiphon* as a separate genus from *Linanthus*, and resolved an array of taxonomic problems; however, several issues remained unclear for *Leptosiphon*. In those studies, sampling of *Leptosiphon* was incomplete, and certain species having strikingly similar floral morphologies appeared non-monophyletic, while other species groups with divergent floral morphologies formed a clade. Our analyses use greater sampling across taxa in *Leptosiphon* and, in addition to nrITS, the non-coding chloroplast region *trnS–trnG* to help resolve the genus phylogeny. These results yielded a more complete phylogeny from which to base further research on floral evolution in this group. However, much work remains to be done in resolving species level relationships. Analyses of morphological character evolution suggest that several characters that have been used to define sections within *Leptosiphon* are misleading. In addition, these data suggest that at least one species (i.e., *L. parviflorus*) might be polyphyletic.

Key Words: California Floristic Province, ITS, *Leptosiphon*, *Linanthus*, Polemoniaceae.

Leptosiphon Benth. (Polemoniaceae) contains 40 taxa (31 species and 9 subspecies), and occurs primarily in western North America, with one species endemic to Chile (*L. pusillus* [Benth.] J.M. Porter & L.A. Johnson) (Bell and Patterson 2000; Porter and Johnson 2000; Patterson and Battaglia 2012). The center of diversity for *Leptosiphon* is in California, where 90% of the species occur in diverse habitats across the California Floristic Province (CFP) and adjacent areas (Bell and Patterson 2000). Species of *Leptosiphon* can be found from low elevation coastal scrub to high elevation desert and montane habitats, with most species common in dry, disturbed soil substrates (Patterson and Battaglia 2012). While most taxa are annuals, four are perennials. Although many species are relatively abundant where they occur, 13 species are listed on the California Native Plant Society's inventory of rare and endangered plants of California (CNPS 2015).

Leptosiphon is distinguished within the Polemoniaceae (but not from all members of *Linanthus* [Benth.]) by opposite, palmately-lobed leaves. *Leptosiphon floribundus* (A. Gray) J.M. Porter & L.A. Johnson subsp. *hallii* (Jeps.) J.M. Porter & L.A. Johnson is the one exception with entire leaves. Corollas are salverform to funnelform, often with a ring of trichomes within the tube (see illustrations in Grant 1959). Stamens are equal in length and attached at the same level within the corolla tube (Porter and Johnson 2000; Patterson and Battaglia 2012). *Leptosiphon* can be difficult to distinguish in the field and in the herbarium, and challenging to specialists and non-specialists alike, because many species and species pairs differ by minute vegetative and/or floral characters.

Polemoniaceae have a long history of taxonomic confusion, at all taxonomic levels (see, for example, discussions in Grant 1959; Wherry 1961; Patterson 1977; Bell et al. 1999; Bell and Patterson 2000; Porter and Johnson 2000). In 1833 (pl. 1622), Bentham described *Leptosiphon* (long corolla tubes, head-like inflorescences, with five species) as distinct from the morphologically similar *Linanthus* Benth. (short corolla tubes, with only one species, *L. dichotomus*), *Fenzlia* Benth. (rotate-campanulate corollas, short corolla tube, and one species, =*Linanthus dianthiflorus* [Benth.] Greene), and *Gilia* Ruiz & Pav. sect. *Dactylophyllum* Benth. (short corolla tube, three species). In 1892, Greene combined the genera of Bentham (including *Leptosiphon*) into *Linanthus*, based primarily on the presence of palmately-lobed opposite leaves (Greene 1892; Milliken 1904).

Although other treatments of these genera were proposed (e.g., Gray [1870] and Brand [1907]; both

included these taxa in *Gilia*), for over 100 years, Greene's inclusion of *Leptosiphon* within a broadly circumscribed *Linanthus* was largely followed (except, for example Jepson 1902; Wherry 1961). Molecular studies from the perspective of phylogenetic relationships challenged Greene's circumscription. Early phylogenies of Polemoniaceae challenged many of the traditional classifications (e.g., Johnson et al. 1996; Porter 1996 [1997]), resulting in renewed efforts to understand the complicated family relationships and taxonomic consequences. Bell (1998), Bell et al. (1999), and Bell and Patterson (2000) conducted the molecular studies focusing on *Linanthus* with nrITS (nuclear) and *matK* (chloroplast), presenting evidence for a nonmonophyletic *Linanthus*. These studies provided compelling evidence to recognize *Leptosiphon* as a distinct genus, separate from *Linanthus*, as Bentham had first described in 1833. Porter and Johnson (2000) formalized the transfer of taxa from *Linanthus* to *Leptosiphon*.

Not only is *Leptosiphon* well-supported as distinct from *Linanthus*, but also *Phlox* L. is well-supported as the sister taxon to *Leptosiphon,* and *Gilia* is the sister taxon to *Linanthus* (Johnson et al. 1996; Porter 1996[1997]; Bell et al. 1999; Bell and Patterson 2000; Johnson et al. 2008; Porter et al. 2010). Additional studies have confirmed recognition of *Leptosiphon* as distinct from *Linanthus*, and that *Gymnosteris*, *Microsteris*, *Phlox*, and *Leptosiphon* form a monophyletic group, with *Linanthus* as the sister taxon to this clade.

PROJECT GOALS

This study represents a publication of thesis work from Hankamp (2012). The primary goal of this project was to use additional molecular data from the chloroplast genome, as well as complete the ITS data set of Bell and Patterson (2000), in order to complete sampling of *Leptosiphon*.

MATERIALS AND METHODS

Taxon Sampling

Sequence data from 70 samples were generated from field-collected plant material and herbarium specimens, or were obtained from GenBank (Table 1). Voucher specimens collected for this project were deposited in the Harry D. Thiers Herbarium at San Francisco State University (SFSU). All but one species of *Leptosiphon* was sampled, including all currently recognized subspecies (Table 1). During this project *L. minimus* (H. Mason) Battaglia material could not be located. In addition to members of *Leptosiphon*, sequences from closely related genera were obtained (e.g., *Phlox*, *Polemonium* L., and *Linanthus*) and used as outgroups based on previous molecular work (Porter 1997; Goodwillie 1999; Johnson 2008). Sample details and GenBank accession numbers for all sequences included in this study are included in Table 1.

DNA Extraction, Amplification, and Sequencing

Genomic DNA was extracted from dried leaves and calyces (herbarium or field material) following Doyle and Doyle (1987) and per the Qiagen DNEasy Plant kits (Qiagen, Valencia, CA, USA).

The non-coding chloroplast region *trnS–trnG* was amplified with the primer pair *trnS* (forward) and *trnG* (reverse) (Hamilton 1998). The region *trnS–trnG* has proven useful in elucidating species-level relationships in studies of closely related species (Hamilton 1998; Shaw et al. 2005; Shaw et. al. 2007; Johnson 2008). Two additional chloroplast regions (*trnL–trnF* and *psbA–trnH*) were also tested in the beginning stages of this project, and did not provide needed resolution for species-level relationships in *Leptosiphon*. We did not go forward with these markers. Amplification in PCR (Mullis et al. 1987) was conducted with a final reaction volume of 25 µl, containing 13.4 µl ultra-pure H_2O, 0.10 µl 10× Exonuclease Taq DNA polymerase (ExTaq) (Takara Bio Inc., Otsu, Shiga, Japan), 2.5 µl ExTaq Mg2+ free buffer, 3.0 µl 25 mM $MgCl_2$, 2.5 µl 10 mM dNTPs, 1.25 µl each of forward and reverse primer at 10 mM, and 1.0 µl genomic DNA template. PCRs reaction thermo-cycle profile had a 2 min at 95°C initial denaturation step, followed by 35 cycles of 30 sec at 94°C, 30 sec at 67°C, 1 min at 72°C, and terminated at 72°C for 5 min extension.

Amplification of the internal transcribed spacer region (ITS-1, ITS-2, and 5.8S gene) of nuclear ribosomal DNA sequences (nrITS) used primers ITS-I (forward) and ITS-4 (reverse) (White 1990; Urbatsch 2000). Amplification in PCR was conducted with a final reaction volume of 25 µl, containing 14.4 µl ultra-pure H_2O, 0.10 µl 10× Exonuclease Taq DNA polymerase (ExTaq) (Takara Bio Inc., Otsu, Shiga, Japan), 2.5 µl ExTaq Mg2+ free buffer, 2.0 µl 25 mM $MgCl_2$, 2.5 µl 10 mM dNTPs, 1.25 µl each of forward and reverse primer at 10 mM, and 1.0 µl genomic DNA template. PCRs reaction thermo-cycle profile had a 2 min at 95°C initial denaturation step, followed by 35 cycles of 30 sec at 94°C, 30 sec at 54°C, 1 min at 72°C, and terminated at 72°C for 5 min extension.

PCR products were cleaned of excess nucleotides from the amplification reaction using 1 µl ExoSAP-IT (USB Corp., Cleveland, OH, USA) per 5 µl template, with an initial 37°C incubation for 30 min for digestion, followed by 80°C for 10 min to inactivate the enzymes. Cycle sequencing for *trnS–trnG* and nrITS used amplification primers. However, several taxa (e.g., *L. rosaceus*, *L. ciliatus*, and *L. pygmaeus* subsp. *pygmaeus*) required a modified internal forward primer ITS-I to get clean sequence, and the primer "ITS-LEU-int" was designed and used for this study (F. Cipriano, personal communication, ITS-LEU-int: 5'-GTAGGTGAACCTGCG

TABLE 1. Voucher and location data, and GenBank accession numbers for the 70 samples from which nrITS and trnS-trnG sequences were obtained. na = sequence was not determined for this accession. Vouchers are deposited at the H. D. Thiers Herbarium of San Francisco State University (SFSU), unless otherwise noted. Localities are from the United States of America (USA), and the state of California (CA), unless otherwise noted. All samples obtained from field material or from herbarium material by PH, or [a] sequences obtained from GenBank for nrITS from Bell et al. (1999), [b] obtained from GenBank for nrITS from Bell and Patterson (2000), [c] dried plant material donated by J. M. Porter (RSA), [d] dried plant material donated by J. Rebman (SDNHM), [e] dried plant material donated by J. Zylstra (RSA).

Genus	Taxon	Voucher	Locality	GenBank accession number ITS	GenBank accession number trnS-trnG
Leptosiphon sect. *Dactylophyllum*					
	L. ambiguus (Rattan) J. M. Porter & L. A. Johnson	P. Hankamp 039	Santa Clara Co.	na	KM986489
	L. ambiguus (Rattan) J. M. Porter & L. A. Johnson[b]	I. de Geofroy 032	Stanislaus Co.	AF119442	na
	L. bolanderi (A. Gray) J. M. Porter & L. A. Johnson	E. A. Swearingen 005	Lake Co.	na	KM986469
	L. bolanderi (A. Gray) J. M. Porter & L. A. Johnson[b]	P. Owings 046	Lake Co.	AF119440	na
	L. chrysanthus J. M. Porter & R. Patt. subsp. chrysanthus[b]	I. de Geofroy 018	San Bernardino Co.	AF119434	na
	L. chrysanthus J. M. Porter & R. Patt. subsp. chrysanthus	P. Hankamp 032	San Bernardino Co.	na	KM986485
	L. chrysanthus J. M. Porter & R. Patt. subsp. decorus (A. Gray) J. M. Porter & R. Patt.	P. Hankamp 031	San Bernardino Co.	na	KM986468
	L. chrysanthus J. M. Porter & R. Patt. subsp. decorus (A. Gray) J. M. Porter & R. Patt.[b]	S. E. Lambert 004	San Bernardino Co.	AF119435	na
	L. filipes J. M. Porter & L. A. Johnson	P. Hankamp 132	Fresno Co.	na	KM986488
	L. filipes J. M. Porter & L. A. Johnson[b]	M. Bourell 3014	Tulare Co.	AF119437	na
	L. harknessii (Curran) J. M. Porter & L. A. Johnson	P. Hankamp 021	Inyo Co.	na	KM986469
	L. harknessii (Curran) J. M. Porter & L. A. Johnson[b]	D. Breedlove 62754	NV, Mineral Co.	AF119433	na
	L. jamauensis (Moran) J. M. Porter & L. A. Johnson[d]	J. Rebman 5308 (SD145290)	Mexico, Baja California	na	KM986510
	L. jamauensis (Moran) J. M. Porter & L. A. Johnson[b]	R. Moran 20930 (CAS)	Mexico, Baja California	AF119436	na
	L. lemmonii (A. Gray) J. M. Porter & L. A. Johnson[d]	L. Hendrickson 2996 (SD209008)	San Diego Co.	na	KM986467
	L. lemmonii (A. Gray) J. M. Porter & L. A. Johnson[a]	M. Bourell et al. 2968 (CAS)	San Diego Co.	AF027694	na
	L. liniflorus (Benth.) J. M. Porter & L. A. Johnson	P. Hankamp 135	San Mateo Co.	na	KM986492
	L. liniflorus (Benth.) J. M. Porter & L. A. Johnson[b]	A. Smyth 029 (SFSU)	San Luis Obispo Co.	AF027695	na
	L. pusillus (Benth.) J. M. Porter & L. A. Johnson	L. A. Johnson s.n.	cultivated at RSABG	AF119439	na
	L. pygmaeus (Brand.) J. M. Porter & L. A. Johnson subsp. continentalis (Raven) J. M. Porter & L. A. Johnson	F. W. Pierson 5924 (RSA73015)	San Diego Co.	KM986501	KM986476
	L. pygmaeus (Brand.) J. M. Porter & L. A. Johnson subsp. pygmaeus[b]	R. Patterson 1915	San Diego Co.	AF119438	na
	L. rattanii (A. Gray) J. M. Porter & L. A. Johnson	P. Hankamp 145	Colusa Co.	na	KM986464
	L. rattanii (A. Gray) J. M. Porter & L. A. Johnson[b]	Smith 8187	Mendocino Co.	AF119441	na
	L. septentrionalis (H. Mason) J. M. Porter & L. A. Johnson[b]	D. Breedlove 62636	NV, Lyon Co.	AF119431	na
Leptosiphon sect. *Leptosiphon*					
	L. androsaceus Benth.[b]	A. Smyth 032	Santa Clara Co.	AF119421	na
	L. aureus Benth. ex E. Vilm.	P. Hankamp 042	Marin Co.	na	KM986506
	L. aureus Benth. ex E. Vilm.	B. Ertter 8904	Alameda Co.	AF119424	na
	L. bicolor Nutt.	P. Hankamp 129	Fresno Co.	na	KM986505
	L. bicolor Nutt.[b]	C. Bell 008	Santa Clara Co.	AF119419	na
	L. breviculus (A. Gray) J. M. Porter & L. A. Johnson	P. Hankamp 045	Los Angeles Co.	na	KM986477
	L. breviculus (A. Gray) J. M. Porter & L. A. Johnson[b]	S. Boyd et al. 1679 (RSA517995)	San Bernardino Co.	AF119425	na

TABLE 1. CONTINUED

Genus	Taxon	Voucher	Locality	GenBank accession number	
				ITS	*trnS-trnG*
	L. ciliatus (Benth.) Jeps.	*P. Hankamp 056*	San Diego Co.	na	KM986483
	L. ciliatus (Benth.) Jeps.	*P. Hankamp 061*	Tuolumne Co.	KM986491	KM986480
	L. ciliatus (Benth.) Jeps.[b]	*D. Breedlove 62833*	El Dorado Co.	AF067546	na
	L. croceus (Eastw.) J. M. Porter & L. A. Johnson	*P. Hankamp 043*	San Mateo Co.	KM986493	KM986482
	L. jepsonii (D. W. Schemske & C. Goodwillie) J. M. Porter & L. A. Johnson[b]	*C. Goodwillie 001*	Napa Co.	AF119420	na
	L. latisectus (E. G. Buxton) J. M. Porter & L. A. Johnson[c]	*G. L. Smith 5446d (RSA396844)*	Mendocino Co.	KM986508	KM986465
	L. montanus (Greene) J. M. Porter & L. A. Johnson	*P. Hankamp 131*	Fresno Co.	KM986464	KM986500
	L. montanus (Greene) J. M. Porter & L. A. Johnson[b]	*S. E. Lambert 023*	Kern Co.	AF119423	na
	L. nudatus (Greene) J. M. Porter & L. A. Johnson[a]	*R. Patterson and R. Zebell 1922*	Kern Co.	AF027692	na
	L. oblanceolatus (Brand) J. M. Porter & L. A. Johnson	*J. M. Keefe 13-662 (CAS)*	Tulare Co.	KM986495	KM986471
	L. parviflorus Benth.	*P. Hankamp 038*	Santa Clara Co.	KM986509	na
	L. parviflorus Benth.	*P. Hankamp 057*	San Diego Co.	KM986513	KM986463
	L. parviflorus Benth.[b]	*R. Patterson 1916*	San Diego Co.	AF119422	na
	L. parviflorus Benth.	*P. Hankamp 059*	Marin Co.	KM986498	na
	L. parviflorus Benth.	*P. Hankamp 060*	Marin Co.	na	KM986463
	L. rosaceus (Hooker) R. Battaglia	*P. Hankamp 044*	San Mateo Co.	KM986504	na
	L. rosaceus (Hooker) R. Battaglia	*R. Stubbs 006*	San Mateo Co.	na	KM986472
	L. serrulatus (Greene) J. M. Porter & L. A. Johnson	*P. Hankamp 130*	Fresno Co.	KM986473	KM986494
Leptosiphon sect. *Pacificus*	L. grandiflorus Benth.	*V. Yadon s.n. (coll. 31 Aug 2013)*	Monterey Co.	na	KM986484
	L. grandiflorus Benth.[a]	*C. Bell s.n. (coll. 01 Sep 1998)*	cultivated at SFSU	AF027692	na
Leptosiphon sect. *Siphonella*	L. floribundus (A. Gray) J. M. Porter & L. A. Johnson subsp. floribundus	*P. Hankamp 058*	San Diego Co.	na	KM986475
	L. floribundus (A. Gray) J. M. Porter & L. A. Johnson subsp. floribundus[b]	*R. Patterson 1912*	San Diego Co.	AF119429	na
	L. floribundus (A. Gray) J. M. Porter & L. A. Johnson subsp. hallii (Jeps.) J. M. Porter & L. A. Johnson[e]	*J. Zylstra 89*	Riverside Co.	KM986497	KM986479
	L. floribundus (A. Gray) J. M. Porter & L. A. Johnson subsp. glaber (R. Patt.) J. M. Porter & L. A. Johnson[d]	*Sweet 658 (SD)*	San Diego Co.	KM986496	KM986481
	L. laxus (Vasey & Rose) J. M. Porter & L. A. Johnson[c]	*J. M. Porter s.n. (coll. 31 Aug 2013)*	Mexico, Baja California	na	KM986486
	L. laxus (Vasey & Rose) J. M. Porter & L. A. Johnson[b]	*R. Moran 25902 (CAS)*	Mexico, Baja California	AF119427	na
	L. melingii (Wiggins) J. M. Porter & L. A. Johnson[d]	*J. Rebman 16018 (SD191994)*	Mexico, Baja California	na	KM986490
	L. melingii (Wiggins) J. M. Porter & L. A. Johnson[b]	*R. Haller 1970*	Mexico, Baja California	AF119428	na
	L. nuttallii (A. Gray) J. M. Porter & L. A. Johnson subsp. howellii (T. W. Nelson & R. Patt.) J. M. Porter & L. A. Johnson	*P. Hankamp 141*	Tehama Co.	KM986503	KM986466
	L. nuttallii (A. Gray) J. M. Porter & L. A. Johnson subsp. nuttallii	*P. Hankamp 063*	UT, Salt Lake Co.	KM986507	na

TABLE 1. CONTINUED

Genus	Taxon	Voucher	Locality	GenBank accession number	
				ITS	trnS-trnG
	L. nuttallii (A. Gray) J. M. Porter & L. A. Johnson subsp. nuttallii[a]	B. Bartholomew & B. Anderson 4295 (CAS)	Modoc Co.	AF027691	na
	L. pachyphyllus (R. Patt.) J. M. Porter & L. A. Johnson	P. Hankamp 062	Tuolumne Co.	na	KM986462
	L. pachyphyllus (R. Patt.) J. M. Porter & L. A. Johnson[b]	R. Patterson and M. Bourell 1803	Inyo Co.	AF119426	na
Linanthus Benth.	Linanthus caespitosus (Nutt.) J.M. Porter & L.A. Johnson[b]	D. Wilken 13982	CO, Moffat Co.	AF119443	na
	Linanthus jonesii (A. Gray) Greene[b]	P. Owings 047	San Bernardino Co.	AF119430	na
Phlox L.	Phlox condensata (A. Gray) E.E. Nelson	P. Hankamp 27	Mono Co.	na	KM986478
	Phlox diffusa Benth.[b]	Peterson 97-110	Sierra Co.	AF119444	na
Polemonium L.	Polemonium viscosum Nutt.[a]	C. Bell 003 (SFSU)	ID, Blaine Co.	AF016051	na

GAAG-3′). The final reaction volume was 12 µl, containing 6.45 µl ultra-pure H20, 0.5 µl BigDye (Applied Biosystems, Inc., Foster City, CA, USA), 2.0 µl 5x buffer, 0.75 µl primer, and 2.0 µl template DNA. Reaction parameters for cycle sequencing were an initial 2 min at 95°C denaturation step, followed by 25 cycles of 20 sec at 94°C, 1 min at 60°C, and extended at 60°C for 4 min. Sequencing was conducted using an ABI PRISM 3100 Sequencer (Applied Biosystems, Inc., Foster City, CA, USA). All molecular work was conducted in the SFSU Department of Biology Genomics/Transcriptomics Analysis Core (GTAC).

Analysis

Sequences were base-called in Sequencing Analysis Software 5.1 (Applied Biosystems, Inc., Foster City, CA, USA). Sequences were edited using Sequencher 4.8 (Gene Codes Corporation, Inc., Ann Arbor, MI). Sequences were aligned using ClustalX (Larkin et al. 2007). All sequences generated for this study were deposited in GenBank (Table 1).

Phylogenetic analysis. All phylogenetic analyses were performed using PAUP* 4.0a145 (Swofford 2002) under the maximum likelihood (ML) criterion. Phylogenetic model selection was completed using PAUP* for 64 models of nucleotide substitution. A models fit was evaluated using Akaike Information Criterion (AIC) values. The results of these analyses indicated the TIM+G model for the *trnS–trnG* data set and SYM+I+G model for ITS data set.

Maximum likelihood searches were conducted using heuristic search methods with tree bisection reconnection (TBR) branch swapping, collapse of zero-length branches, and all characters weighted equally. The analyses were repeated 100 times with the RANDOM ADDITION option. Sets of equally most parsimonious trees were summarized with a strict consensus tree. Bootstrap tests (Felsenstein 1985) were performed using 300 replicates with heuristic search settings identical to those of the original search.

Many of our sequences were not obtained from the same specimen/accession (see Table 1). Combining data from such a sampling scheme could lead to incongruence among gene trees because of paraphyletic or, worse, polyphyletic species. Because of this, we did not perform a combined analysis of our data.

Character state reconstruction. To evaluate the evolutionary history of several morphological characters that have been used to diagnose relationships among species within *Leptosiphon* (namely the presence of thread-like pedicels, and calyx membrane width relative to calyx lobe width), we estimated ancestral character states using maximum likelihood (ML). We selected among three different transition probability matrices: 1) a one-parameter equal rates model (ER), 2) a symmetric model (SYM) in which

forward and reverse transitions are constrained to be equal, and 3) an all rates different (ARD) where all transitions between characters states has a unique parameter. A likelihood ratio test was then used to evaluate the fit of each of the models. All character state reconstructions were performed using the APE module (Paradis 2006) for the R statistical computing platform.

All character state reconstructions were done on the ML tree topology from the ITS data because it included the most taxa. Prior to these analyses, branch lengths were parameterized using the penalized likelihood (PL) method of Sanderson (2002) and implemented in APE (Paradis 2006). In addition, outgroup taxa (i.e., *Polemonium*, *Phlox*, and *Linanthus*) were pruned from the tree for all character state analyses.

RESULTS

Phylogenetic analyses of trnS–trnG *region data.* Of the 557 included characters, 306 are constant, 80 variable characters are parsimony-uninformative, and 171 characters are parsimony-informative. Sequence length heterogeneity was present with a range of 580–711 base pairs (bp) for the fragments amplified. Consistent but arbitrary starting and ending points were selected within the aligned dataset for all model testing. This ensured that regardless of actual number of bases sequenced per species, all taxa in the dataset were analyzed using the same number of aligned base pairs.

The heuristic search recovered 2 trees with identical negative log-likelihood scores of 1851.11. One of these trees is presented with bootstrap values in Figure 1.

Relationships among members of *Leptosiphon* were largely unresolved for the *trnS–trnG* tree, and there was no support for any of Grant's (1959) sections. However, some derived groupings were significantly supported, and they can be used to corroborate the arrangement presented by the ITS region analysis (Fig. 2). For example, the *L. liniflorus* and *L. grandiflorus* clade was well supported in both analyses, despite the fact that these species belong to completely different sections.

Phylogenetic analyses of nrITS data. A 650 bp fragment was sequenced for the ITS-1, 5.8S, and ITS-2 regions. Previous researchers have reported the boundaries of these regions within the overall ITS region (Porter 1997; Bell and Patterson 2000), and the sequences generated by this project closely matched the published demarcations for ITS-1 (248–253 bp), 5.8S (164 bp), and ITS-2 (221–228 bp). Because of the absence of variable sites within the 5.8S region, the sequences from this region were excluded from all analyses.

Of the remaining sequences from both ITS-1 and ITS-2, 232 characters are constant, 63 variable characters are parsimony-uninformative, and 106 characters are parsimony-informative. Sequence length heterogeneity was present with a range of 540–650 bp for the fragments amplified. Aligned sequence for all taxa was trimmed as to be homogenous in length for analysis, as described for the *trnS–trnG* region.

The heuristic search recovered a single tree with a negative log-likelihood score of 2595.26 (Fig. 2). Bell and Patterson (2000) were unable to amplify and sequence several species (*L. serrulatus*, *L. oblanceolatus*, *L. latisectus*, *L. rosaceus*, *L. croceus*), and several subspecies (*L. pygmaeus* subsp. *pygmaeus*, *L. floribundus* subsp. *glaber*, *L. floribundus* subsp. *hallii*, *L. nuttallii* subsp. *pubescens*, *L. nuttallii* subsp. *howellii*). This project was able to obtain sequences for all of the aforementioned taxa (Fig. 2).

Complete sampling of all members of Grant's 1959 sections did not support the recognition of either sect. *Dactylophyllum* or sect. *Leptosiphon*. Section *Dactylophyllum* does not appear monophyletic, but several subgroups are well supported based on our bootstrap analyses. Section *Leptosiphon* was missing five species in previous analyses, but complete sampling did not support it as a monophyletic group. Section *Siphonella* (mostly perennials with one Baja California annual) is supported as monophyletic after the addition of four subspecies.

Character state reconstruction. We could not reject the simpler equal rates model for each of the two characters (thread-like pedicels and calyx membrane narrower than sepals) based on a Chi-square test (both $P > 0.05$). Both characters show some degree of homoplasy and reconstructions of each are presented in Figures 3 and 4.

DISCUSSION

One goal of our project was to investigate the sectional integrity of *Leptosiphon*. Grant (1959) recognized six sections within *Linanthus* based on morphological features. Four of these sections are currently in *Leptosiphon* (sects. *Dactylophyllum*, *Leptosiphon*, *Pacificus*, and *Siphonella*), and the other two (sects. *Dianthoides* and *Linanthus*) were retained in *Linanthus*. Recent authors have added to Grant's treatment, and are noted below in the section descriptions. Grant's sections follow.

I. Section *Dactylophyllum* (Benth.) Grant

Members of this section are annuals characterized by filiform stems and leaf lobes, and flowers on thread-like pedicels (Fig. 3), except *L. lemmonii*, which has sessile flowers. There are eleven North American annual species (*L. ambiguus*, *L. chrysanthus*, *L. bolanderi*, *L. filipes*, *L. harknessii*, *L. lemmonii*, *L. liniflorus*, *L. pusillus*, *L. pygmaeus*, *L. rattanii*, *L. septentrionalis*), with ranges as far north as Washington and as far south as Baja California. Most are endemic to parts of the California Floristic Province. An additional species, *L. pusillus*,
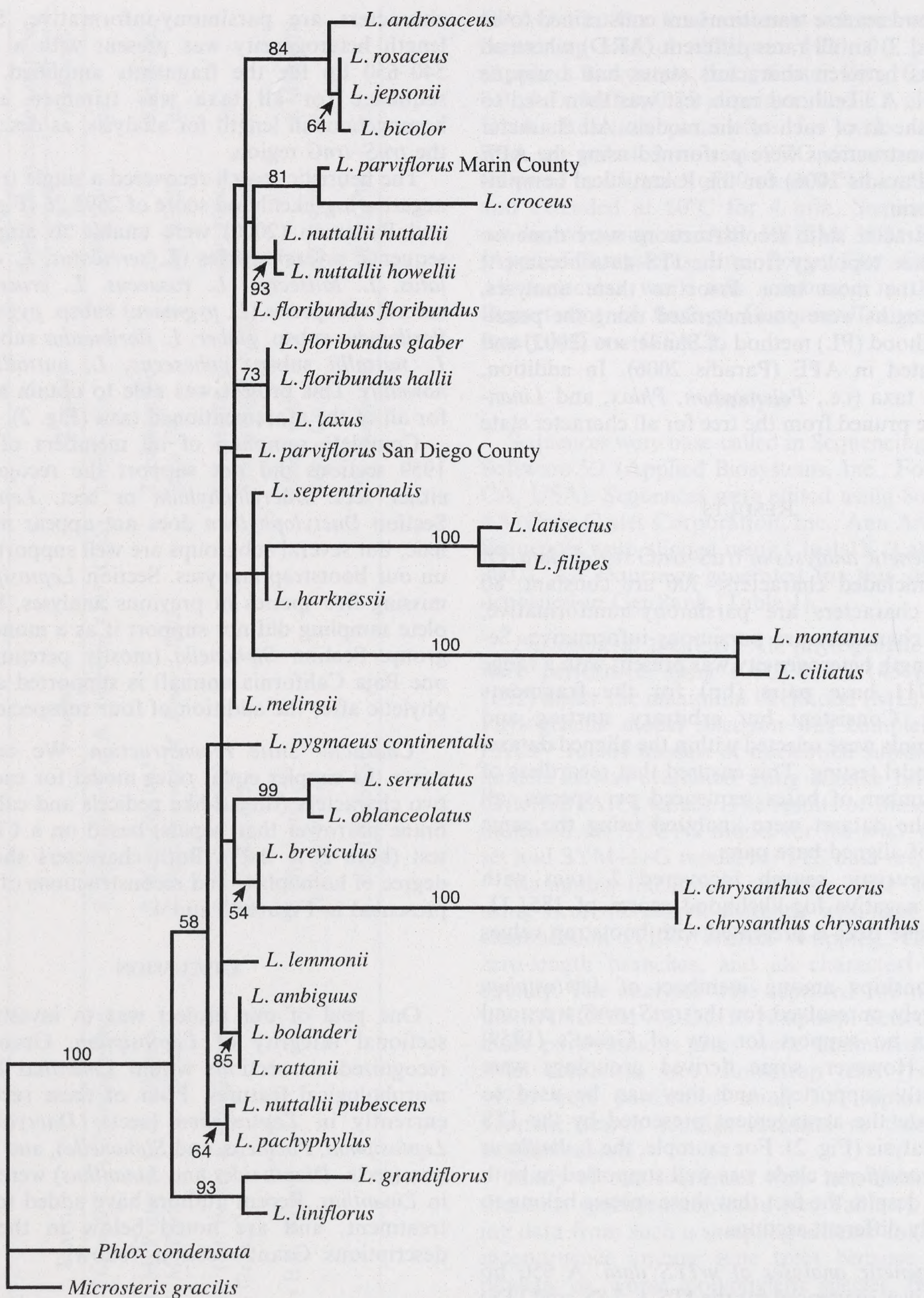
FIG. 1. Maximum likelihood phylogram for trnS-trnG dataset. −lnL=1869.28. Values above branches represent bootstrap values >50%.

is native to Chile. Recently, Porter and Patterson (2015) reviewed the nomenclatural history of *L. aureus*, and uncovered an error in synonymy. That name was earlier applied to what has been referred to as *L. acicularis*, requiring this common desert species to have a new epithet. What has been known as *L. aureus* is now named *L. chrysanthus* J.M.Porter & R. Patt. Likewise, the name *L. aureus* is

retained but is applied to the species formerly called *L. acicularis*.

The only detailed study of members of this section was conducted by (Hiss 1996). Her morphometric analysis of *L. bolanderi*, *L. ambiguus*, and *L. rattanii* (the *L. bolanderi* complex) concluded that there was minimal morphological variation to distinguish *L. bolanderi* and *L. rattanii*; however, the serpentine
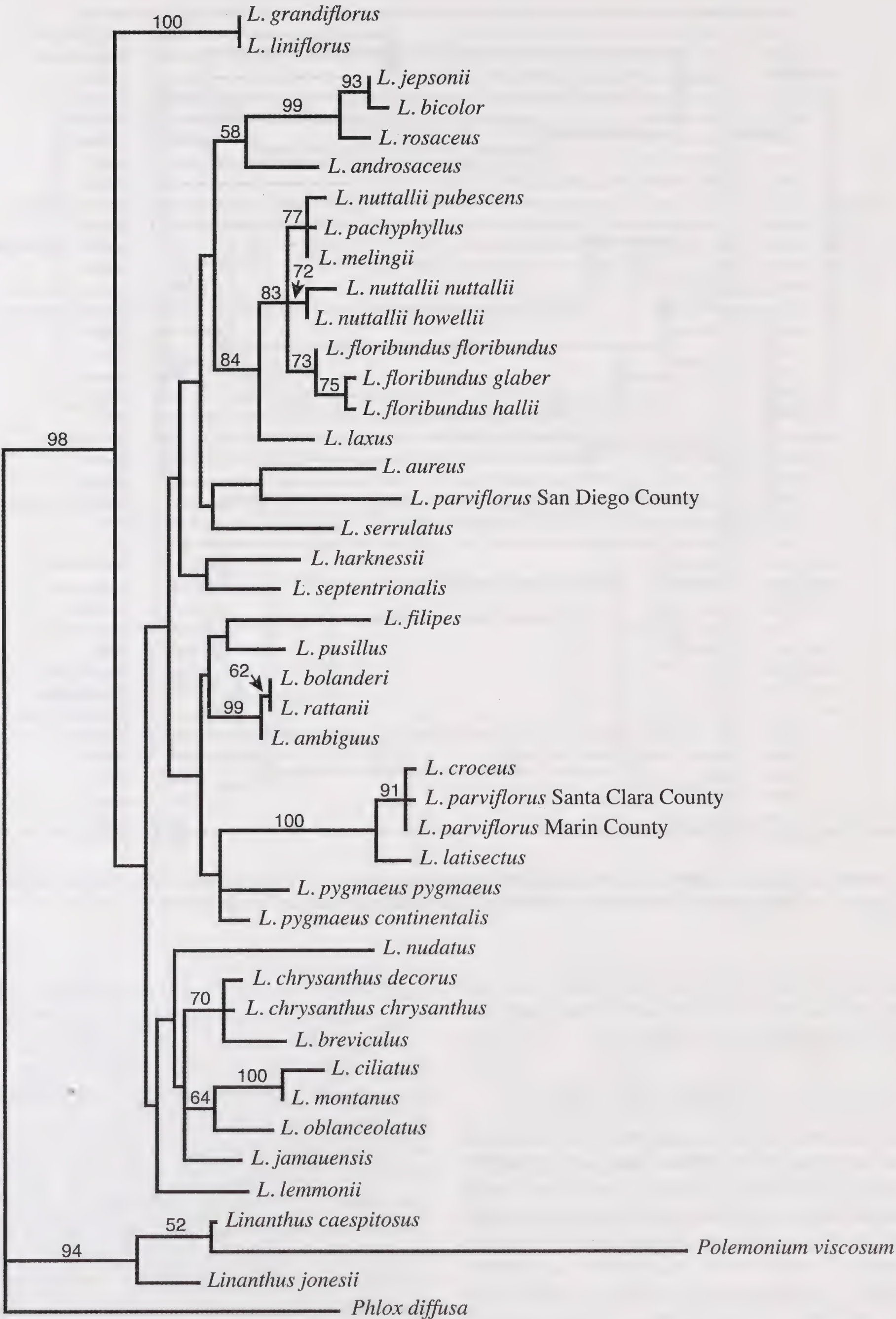
FIG. 2.   Maximum likelihood phylogram for ITS dataset. −lnL=2629.91. Values above branches represent bootstrap values >50%.

near-endemic, *L. ambiguus* was clearly distinct morphologically.

Section *Dactylophyllum* sensu Grant is not monophyletic based on the ITS sequence data (Fig. 3). *Leptosiphon bolanderi*, *L. rattanii*, *L. ambiguus*, *L.* *pusillus, and L. filipes* form a monophyletic group, as do *L. harknessii* and *L. septentrionalis*. However, *Leptosiphon liniflorus, L. pygmaeus, L. chrysanthus, L. jamauensis*, and *L. lemmonii* are each sister to different species from other sections. Multiple
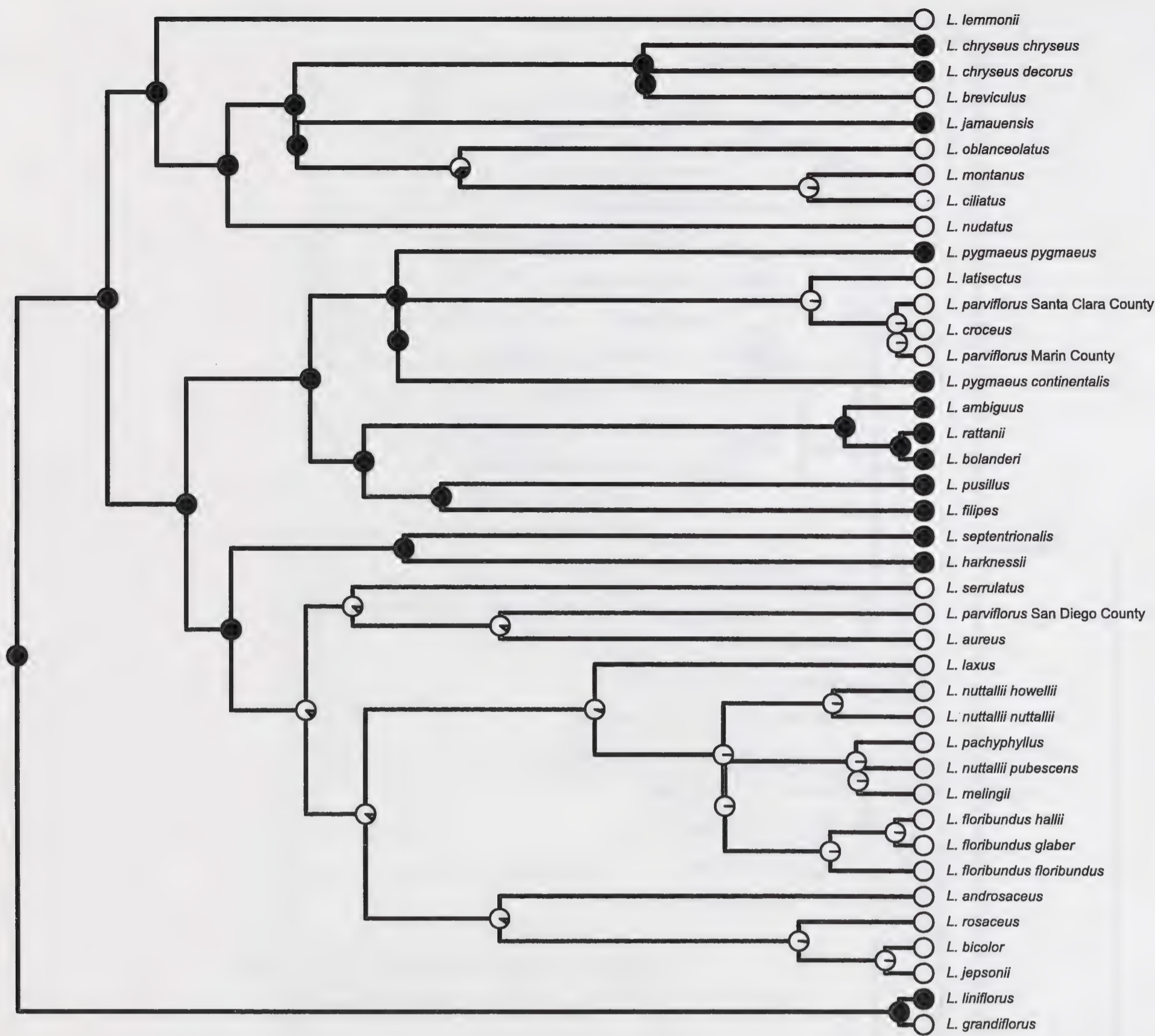
FIG. 3. Presence (black) of flowers on thread-like pedicels mapped onto ITS region gene tree (shown by dashed lines). Circles at internal nodes represent marginal probability of each character state for that node.

populations of *L. liniflorus* were sampled (data not presented) to ensure that it forms a clade with *L. grandiflorus* (monotypic sect. *Pacificus*).

II. Section *Leptosiphon* (Endl.) Grant

Grant characterized this section by presence of salverform corollas with tubes that are generally twice as long as the calyx (except *L. serrulatus*), with sessile flowers borne in dense heads. Grant assigned ten annuals to this section (*L. aureus* [which he called *L. acicularis*], *L. androsaceus*, *L. bicolor*, *L. breviculus*, *L. ciliatus*, *L. montanus*, *L. nudatus*, *L. oblanceolatus*, *L. parviflorus*, *L. serrulatus*), which occur in diverse habitats throughout western North America. Two additional species have since been described: *L. latisectus* (Buxton 1994) and *L. jepsonii* (Schemske and Goodwillie 1996), and three subspecies have been elevated to species rank: *Leptosiphon rosaceus* and *L. minimus* (Battaglia and Patterson 2001), and *L. croceus* (Porter and Johnson 2000).

Despite all but one member of this group being included in the analysis, sect. *Leptosiphon* sensu Grant is not monophyletic based on the ITS

sequence data (Fig. 2). The corolla and inflorescence pattern appears to have evolved several times.

Within this group, ten of the fifteen species possess calyx lobes that are much wider than the nearly obscure calyx membranes that connect the lobes (Fig. 4). This *L. androsaceus* alliance is not monophyletic, with three distinct clades each sister to parts of other sections. The other five species in this group, *L. breviculus*, *L. ciliatus*, *L. montanus*, *L. nudatus*, and *L. oblanceolatus* possess calyx membranes as wide as calyx lobes. These species form part of a monophyletic group, which includes *L. chrysanthus* and *L. jamauensis*. It remains a mystery why these latter species group with the former in the ITS tree, but it appears that calyx membrane width alone does not help to distinguish monophyletic groups in this genus.

Goodwillie and Stiller (2001) discussed the utility of pubescence features in *Leptosiphon*, pointing out that trichome density and glandularity has served to help distinguish species in the genus. Presence or absence of glandular trichomes on calyces is particularly useful in recognizing members of the *L.*
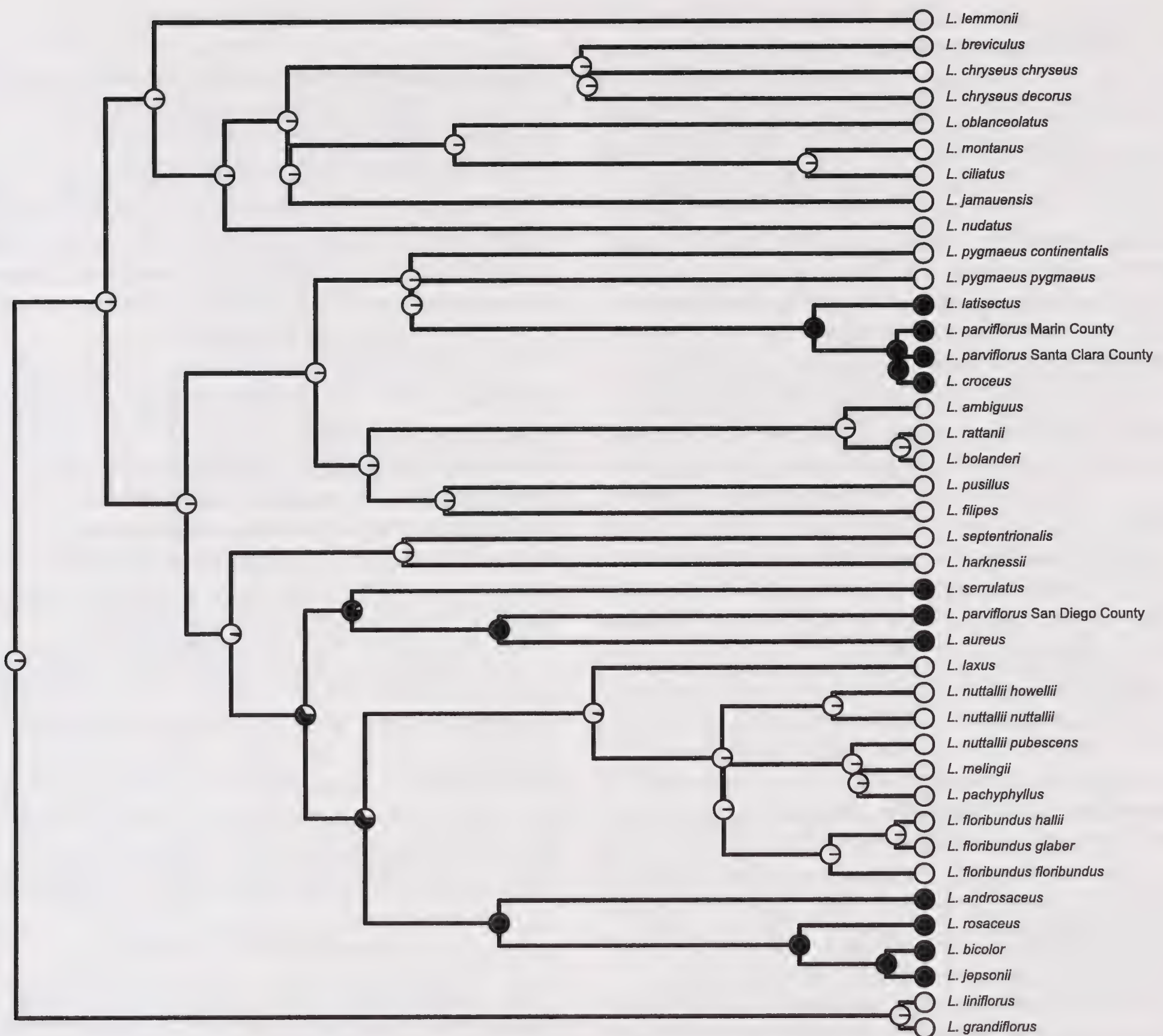
FIG. 4.   Presence of calyx membrane narrower than sepals (black) mapped onto ITS region gene tree (shown by dashed lines). Circles at internal nodes represent marginal probability of each character state for that node.

androsaceus complex. The clade consisting of *L. croceus*, *L. parviflorus* (Marin County and Point Reyes), and *L. latisectus* has glandular calyces, while the clade comprising *L. androsaceus*, *L. bicolor*, *L. jepsonii*, and *L. rosaceus* lack glandular calyx trichomes. A third clade exists comprising *Leptosiphon aureus* (=*acicularis*) plus *L. parviflorus* (San Diego County), both with glandular calyces, and *L. serrulatus*, with non-glandular calyces.

Leptosiphon parviflorus exhibits a particularly high amount of corolla variability in lobe width, color, and color patterns. Although originally described by Bentham (1833) at the same time as *L. androsaceus*, it was subsumed taxonomically into *L. androsaceus* by Grant (1959), and thus largely ignored by more recent botanists. Yet, as many as eight described taxa have been ascribed to *L. parviflorus*, and two of these, *L. croceus* and *L. rosaceus*, have been promoted to species rank. *Leptosiphon parviflorus* remains understudied and although our research included three samples from disparate populations, a much more thorough sampling across its range in California is needed.

III. Section *Pacificus* (Jeps.) Grant

This section consists of only the annual *L. grandiflorus*. The combination of sessile flowers with funnelform corollas in terminal heads distinguishes *L. grandiflorus* from the rest of the genus. It is strongly supported on the ITS tree as sister to *L. liniflorus*, with the pair sister to the remainder of the genus. Although their ITS relationship is compelling, these two species share very few morphological characters, and future studies will be needed to clarify this relationship.

IV. Section *Siphonella* (Gray) Grant

Grant recognized three species within this section (*L. laxus*, *L. melingii*, *L. nuttallii*) based primarily on perennial habit (except *L. laxus*), and funnelform corollas with short tubes, more or less equal in length to the calyx lobes. Patterson (1977) recognized two additional perennials in this section, *L. floribundus* and *L. pachyphyllus*. *Leptosiphon melingii* and *L. pachyphyllus* are tetraploids, apparently the only polyploids in the genus. These five species form a reasonably strong clade, although support is lower for relation-

ships within the section. The perennial habit may be the most reliable morphological feature in the genus.

Most of the currently recognized sections of *Leptosiphon* were not supported using the ITS and *trnS–trnG* regions (Figs. 1 and 2), with the former providing more resolution for the phylogeny of *Leptosiphon*. Despite this lack of support for Grant's sectional taxonomy, many of the relationships among taxa inferred on the ITS and *trnS–trnG* gene trees support the hypotheses of previous researchers. In addition, there is strong support for the phylogenetic placement of previously unsampled species (*L. rosaceus*, *L. croceus*), as well as several subspecies (*L. floribundus* subsp. *glaber* and subsp. *hallii*, *L. nuttallii* subsp. *howellii* and subsp. *pubescens*). At the same time, the regions ITS and *trnS–trnG* are of limited utility in their ability to resolve deeper level relationships among the sections. In phylogenetic analyses of both datasets, sections *Leptosiphon* and *Dactylophyllum* remain non-monophyletic. Section *Siphonella* remains monophyletic, and shows even more significant support for branches after greater sampling. Both datasets from this project show high bootstrap support for several interspecific relationships. However, these regions did a poor job of resolving the basal branches, suggesting that *Leptosiphon* species diversified rapidly, not allowing mutations to be fixed in the genome. Also, sequence ambiguity in the ITS region, and especially in the *trnS–trnG* region, contributed to a lack of resolution for most basal nodes on the phylogenetic trees.

Section *Siphonella* (mostly perennials with one Baja California annual) remains monophyletic after the addition of four subspecies (Fig. 2). Grant (1959) placed the annual *L. laxus* with the perennials based on calyces with long lobes and narrow hyaline membranes, as well as a stout corolla tube (Patterson 1977). The phylogeny for the ITS gene tree corroborates Grant's classification, with *L. laxus* sister to the perennials. This study included the subspecies *L. floribundus* subsp. *glaber* and *hallii* and *L. nuttallii* subsp. *howellii* and *pubescens*, and the result was a highly supported arrangement of a monophyletic section on the ITS gene tree. *Leptosiphon nuttallii* subsp. *pubescens* forms a clade with *L. pachyphyllus* and *L. melingii* (a narrow endemic from a mountain in Baja California), and not with the other two subspecies of *L. nuttallii*.

Patterson (1980) tested hybridization among all members of sect. *Siphonella* and found that some interspecific crosses result in a low percent of viable seeds. Patterson concluded that interpopulational genomic variation at the chromosome level does occur, and in an evolutionary timescale, *L. pachyphyllus* may have arisen via hybridization from geographically similar populations with different chromosome numbers. Given these results, it is not surprising that *Leptosiphon nuttallii* subsp. *pubescens* forms a clade with *L. pachyphyllus* on the gene tree presented here, since they both share an overlapping bioregional distribution in the eastern Sierra Nevada near the same elevation.

CONCLUSIONS

These analyses, as well as others (e.g., Bell and Patterson 2000), call into question the value of many of the morphological characters that have been used to circumscribe infrageneric sections and estimate relationships in *Leptosiphon* and in Polemoniaceae. While traditionally used morphological features currently serve to differentiate species of *Leptosiphon*, many appear as homoplasies in molecular phylogenies and deserve reconsideration.

It is also obvious that phylogenetically informative sequence data is needed to further resolve the phylogeny and relationships of this group. Using the latest generation sequencing methods to obtain large amounts of genomic sequence data would be a logical next step. With more genomic sequence data, several different phylogenetic methods, such as multispecies coalescent and Bayesian Concordance analyses, can be employed for this group.

In addition, to further explore character evolution or biogeography in this group, more geographic and ecological data will be needed. Ecological niche modeling could be used here to determine if environmental variables are influencing mating system evolution and maintenance within this group. In line with this, more field observations of mating systems will be necessary to rule out plasticity.

LITERATURE CITED

BATTAGLIA, R. E. AND R. PATTERSON. 2001. A morphometric analysis of the *Leptosiphon androsaceus* complex (Polemoniaceae) in the central and south Coast Ranges. Madroño 48:62–78.

BELL, C. D. 1998. Molecular phylogeny of *Linanthus* (Polemoniaceae). M.A. Thesis, Biology, San Francisco State University, San Francisco, CA.

——— AND R. PATTERSON. 2000. Molecular phylogeny and biogeography of *Linanthus* (Polemoniaceae). American Journal of Botany 87:1857–1870.

———, ———, AND L. A. HAMILTON. 1999. Sectional integrity in *Linanthus* (Polemoniaceae): a molecular phylogeny of section *Dianthoides*. Systematic Botany 24:632–644.

BENTHAM, G. 1833. *Collomia coccinea*, t. 1622, with descriptions for *Hugelia*, *Linanthus*, *Leptosiphon*, *Fenzlia*, *Gilia*, *Aegochloa*, and *Collomia* in J. Lindley (ed.), Edwards's botanical register, or, Ornamental flowergarden and shrubbery: consisting of coloured figures of plants and shrubs, cultivated in British gardens; accompanied by their history, best method of treatment

in cultivation, propagation, &c. Vol. 19 (new series Vol. 6). James Ridgway, London.

BERGSTEN, J. 2005. A review of long-branch attraction. Cladistics 21:163–193.

BUXTON, E. G. 1994. *Linanthus latisectus* (Polemoniaceae), a newly described species from the California North Coast Ranges. Madroño 41:178–184.

CALIFORNIA NATIVE PLANT SOCIETY (CNPS). 2015. Inventory of rare and endangered plants (online edition, v8-02). California Native Plant Society, Sacramento, CA. Website http://www.cnps.org/inventory (accessed 01 June 2015).

DOYLE, J. J. AND J. L. DOYLE. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

GOODWILLIE, C. 1999. Multiple origins of self-compatibility in *Linanthus* section *Leptosiphon* (Polemoniaceae): phylogenetic evidence from internal-transcribed-spacer sequence data. Evolution 53:1387–1395.

———— AND J. W. STILLER. 2001. Evidence for polyphyly in a species of *Linanthus* (Polemoniaceae): convergent evolution in self-fertilizing taxa. Systematic Botany 26:273–282.

GRANT, V. 1959. Natural history of the phlox family. Martinus Nijhoff, The Hague.

GREENE, E. L. 1892. Some American Polemoniaceae, II. Pittonia 2:251–260.

HAMILTON, M. B. 1998. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Molecular Ecology 8:521–523.

HANKAMP, P. Z. 2012. Molecular systematics of *Leptosiphon* (Polemoniaceae). M.S. Thesis, Biology, San Francisco State University, San Francisco, CA.

HISS, A. E. 1996. A biosystematic study of the *Linanthus bolanderi* (Polemoniaceae) complex. M.A. Thesis, San Francisco State University, San Francisco, CA.

HOLDER, M. AND P. O. LEWIS. 2003. Phylogeny estimation: traditional and Bayesian approaches. Nature Reviews in Genetics 4:275–285.

JEPSON, W. L. 1902. A school flora for the Pacific coast. D. Appleton and Co., New York, NY.

JOHNSON, L. A., J. L. SCHULTZ, D. E. SOLTIS, AND P. S. SOLTIS. 1996. Monophyly and genetic relationships of Polemoniaceae based on *matK* sequences. American Journal of Botany 83:1207–1224.

————, L. M. CHAN, T. L. WEESE, L. D. BUSBY, AND S. MCMURRY. 2008. Nuclear and cpDNA sequences combined provide strong inference of higher phylogenetic relationships in the phlox family (Polemoniaceae). Molecular Phylogenetics and Evolution 48:997–1012.

LARKIN, M. A., G. BLACKSHIELDS, N. P. BROWN, R. CHENNA, P. A. MCGETTIGAN, H. MCWILLIAM, F. VALENTIN, I. M. WALLACE, A. WILM, R. LOPEZ, J. D. THOMPSON, T. J. GIBSON, AND D. G. HIGGINS. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948.

MADDISON, D. R. AND W. P. MADDISON. 2005. MacClade 4: Analysis of phylogeny and character evolution. Version 4.08 for OSX. Sinauer Associates, Sunderland, MA.

MILLIKEN, J. 1904. A review of California Polemoniaceae. University of California Publications in Botany 2:1–171.

MORAN, R. 1977. New or renovated Polemoniaceae from Baja California, Mexico (*Ipomopsis, Linanthus, Navarretia*). Madroño 24:141–159.

MUNZ, P. A. AND D. D. KECK. 1959. A California flora with supplement. University of California Press, Berkeley, CA.

PARADIS, E. 2006. Analysis of phylogenetics and evolution using R. Springer, New York, NY.

PATTERSON, R. 1977. The generic status of perennial species of *Linanthus* (Polemoniaceae). Taxon 26:507–511.

————. 1980. Crossing relationships in perennial *Linanthus* taxa (Polemoniaceae). Systematic Botany 5:384–390.

———— AND R. BATTAGLIA. 2012. *Leptosiphon*, pp. 1058–1060 *in* B. G. Baldwin, D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti, and D. H. Wilken (eds.), The Jepson Manual: Vascular plants of California, 2nd ed. University of California Press, Berkeley, CA.

PORTER, J. M. 1996 [1997]. Phylogeny of the Polemoniaceae based on nuclear internal transcribed spacer DNA sequences. Aliso 15:57–77.

———— AND L. A. JOHNSON. 2000. A phylogenetic classification of Polemoniaceae. Aliso 19:55–91.

———— AND R. PATTERSON. 2015. A fistful of Polemoniaceae: new names and combinations. Aliso 32:55–88.

————, L. A. JOHNSON, AND D. WILKEN. 2010. Phylogenetic systematics of *Ipomopsis* (Polemoniaceae): Relationships and divergence times estimated from chloroplast and nuclear DNA sequences. Systematic Botany 35:181–200.

SANDERSON, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. Molecular Biology and Evolution 19:101–109.

SCHEMSKE, D. W. AND C. GOODWILLIE. 1996. Morphological and reproductive characteristics of *Linanthus jepsonii* (Polemoniaceae), a newly described, geographically restricted species from northern California. Madroño 43:453–463.

SEQUENCHER® VERSION 4.8 SEQUENCE ANALYSIS SOFTWARE. 2007. Gene Codes Corporation. Ann Arbor, MI. http://www.genecodes.com.

SEQUENCING ANALYSIS® VERSION 5.1. 2003. Applied Biosystems USA. http://www.appliedbiosystems.com.

SHAW, J., E. B. LICKEY, E. E. SCHILLING, AND R. L. SMALL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. American Journal of Botany 94:275–288.

————, ————, J. T. BECK, S. B. FARMER, W. LIU, J. MILLER, K. C. SIRIPUN, C. T. WINDER, E. E. SCHILLING, AND R. L. SMALL. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. American Journal of Botany 92:142–166.

SWOFFORD, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0 beta 10. Sinauer Associates, Sunderland, MA.

URBATSCH, L. E., B. G. BALDWIN, AND M. J. DONOGHUE. 2000. Phylogeny of coneflowers and relatives (Heliantheae: Asteraceae) based on nuclear rDNA internal transcribed spacer (ITS) sequences and chloroplast DNA restriction site data. Systematic Botany 25:539–565.

WHERRY, E. T. Two linanthoid genera. American Midland Naturalist 34:381–387.

WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal genes for phylogenetics, pp. 315–322 *in* M. A. Innis, D. H. Gelfand, J. J. Sinisky, and T. J. White (eds.), PCR protocols: a guide to methods and applications. Academic Press, New York, NY.

A REVALUATION OF THE TAXONOMIC STATUS OF *HAPLOPAPPUS RAVENII* USING MORPHOLOGICAL AND GENETIC DATA

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ABSTRACT

Slender goldenweed (*Xanthisma gracile*; synonym *Haplopappus gracilis*) ($2n = 4$) is an annual, highly polymorphic, and taxonomically controversial species occurring in the southwestern United States and northern Mexico. It is very similar in morphology to *Haplopappus ravenii*, but differs in chromosome number, with *H. ravenii* having $2n = 8$. Some botanists have considered these to be separate species in the past, but *H. ravenii* has not been recognized in the most recent taxonomic treatments of this complex. Numerous researchers have questioned the morphological distinctiveness of these two taxa, but no study has quantified differences in morphology across geographically diverse populations or tested for genetic differentiation between these taxa. The difference in chromosome number suggests that these taxa could be reproductively isolated, which would support recognition of these as unique taxa under the biological species concept. Here, we applied an alternative species concept, the genotypic cluster criterion to test the hypothesis that *X. gracile* and *H. ravenii* are distinct species and warrant recognition at this level. Flower and leaf characters were measured on herbarium specimens and genetic diversity was quantified using AFLP's. Little evidence of morphological differences in samples assigned to each species was found as no characters exhibited significantly different means. Genetic differentiation between the two groups of samples was significant in an analysis of molecular variance, but the level of divergence was quite low ($\phi_{ST} = 0.015$, $P < 0.05$) in comparison to values for other clearly demarcated species. Clustering analysis did not indicate cleanly separated groups of morphologically or genetically divergent samples. Although this study did not test for reproductive isolation, little evidence was found to indicate that these taxa are divergent, an expected outcome if these species are not exchanging genes. We suggest that *X. gracile* is a polymorphic species with a varied distribution of cytotypes and should continue to be recognized as a single species.

Key Words: AFLP, Asteraceae, genetic divergence, genotypic cluster criterion, *Haplopappus ravenii*, taxonomy, *Xanthisma gracile*.

Karyotypic diversity in number, size, and organization of chromosomes between and within taxa has been discovered in a multitude of cytological analyses since the discovery of chromosomes (Levin 2002). The theory of chromosomal speciation proposes that changes in chromosomal structure or arrangement may cause reproductive isolation between sister taxa and lead to subsequent speciation (White 1968). Cytotaxonomists often give more credence to cytological characters, such as change in chromosome numbers or rearrangements, than traditional morphological characters, because cytological differences are strong barriers to reproduction for many taxa. According to Löve (1960), "the chromosomes determined the characters and not the other way around." However, changes in cytological characters may be overemphasized in the delimitation of some species, given that many species are suspected of having an allopolyploid history and triploid bridges often allow different cytotypes within a species to successfully interbreed (reviewed in Ramsey and Schemske 1998). The use of multiple data sets, including genetic markers, morphology, and ecology, in addition to cytological data, helps in providing robust support to taxonomic revisions and avoids

potential bias associated with the use of a single data type. This approach has been termed as integrative taxonomy (Schlick-Steiner et al. 2010; Padial et al. 2010; Barrett and Freudenstein 2011; Cruz-Barraza et al. 2012) and is rapidly gaining credence in addressing such hypotheses.

An excellent example where difference in karyotype has been attributed to a separate taxonomic status is in *Xanthisma* DC. (Asteraceae), specifically, *Xanthisma gracile* (Nutt.) D.R. Morgan & R.L. Hartman. This species was originally identified as *Haplopappus gracilis* (Nutt.) A. Gray, but the taxonomy of this group has undergone numerous changes recently, and it is currently recognized within *Xanthisma*. The current name will be used throughout this manuscript.

Xanthisma gracile is a small annual species (5–100 cm in height) with the lowest reported chromosome number in angiosperms ($n = 2$; Jackson 1957). It has erect pubescent stems, basal and cauline pinnatifid leaves, and yellow radiate heads that are borne singly on leafy peduncles (Morgan and Hartman 2003). This species occurs principally in the southwestern United States and some regions of northern Mexico (Jackson 1962). *Xanthisma gracile* is a chromosom-

ally polymorphic species with $2n$ numbers of 4, 5, 6, and 7 (Jackson 1960b), although populations are predominantly $2n = 4$ (Jackson 1965). Populations with $2n = 6$ are limited to some areas of New Mexico, and plants with $2n = 5$ appear to be hybrids because they are found only where $n = 2$ and $n = 3$ plants come into contact in some parts of south central Arizona (Jackson 1960b).

Plants with $2n = 8$ were first collected by Peter Raven (Raven et al. 1960) from Yavapai County, AZ and Zion National Park, UT and named as a different species, *Haplopappus ravenii* Jackson (note that this combination will be used throughout the manuscript because the current taxonomy for *Xanthisma* does not include this as a distinct species; Morgan and Hartman 2003). *Haplopappus ravenii* is morphologically very similar to *X. gracile,* and until a greenhouse experiment conducted by Jackson (1962), they were recognized under a single species concept (Hall 1928; Kearney and Peebles 1951; Raven et al. 1960). The main characters used by Jackson (1962) to differentiate the two taxa were the pappus and achene lengths: longer and narrower in *X. gracile,* and phyllary pubescence: appressed in *X. gracile* and stiff in *H. ravenii.* Hybrids were found to be vigorous, intermediate in morphology for the characters differentiating the parent taxa, but with reduced pollen fertility (5 plants) at an average of 6.9%. Evidence was also provided for *X. gracile* to be an aneuploid derived from "*H. ravenii* or a very similar taxon" (Jackson 1962, 1965). Cytological data from F1 hybrids between the two taxa showed that the two chromosomes of *X. gracile* completely synapse with the four of *H. ravenii* (Tanaka 1967).

Jackson and Crovello (1971) stated that *X. gracile* has at least three morphological races, each existing in a different habitat. One of these occurs in the desert grasslands of Arizona and Sonora, Mexico, the second in the dry foothills surrounding areas where the first race occurs in Arizona, and the third in mesic habitats, such as the pinyon juniper woodlands of Arizona, northern New Mexico, and southern Colorado, and in the arid grasslands and savannas in southeastern Arizona, southern New Mexico, northwestern Texas, and western Sonora, Mexico. Two races were also proposed for *H. ravenii*: the arid California race occurring in the mountains of San Bernardino County and the mesic race of Utah and Arizona (Jackson 1965). After evaluating 31 different morphological characters, Jackson and Crovello (1971) summarized that, along with the different chromosome numbers, morphological traits such as the phyllary pubescence, achene and pappus characters, and aspects of leaves, supported the recognition of *H. ravenii* as distinct from *X. gracile.* However, these inferences were based on data from only two populations of *H. ravenii* from Utah and California. They asserted that phyllary pubescence alone would be able to 100% confidently differentiate the two taxa, whereas increasing the characters to 29–31 blurred the boundaries between species.

The separation of *H. ravenii* as a species was questioned by Cronquist (1971), who instead treated it as a single cytologically polymorphic species and noted that the difference in cytology was not "taxonomically controlling." However, later studies, such as the work of Matos (1979), in characterizing the morphological and genetic divergence of the two *H. ravenii* races, identified high genetic divergence between populations, and no hybrids or viable seed sets between the Utah and California races, which suggests of the presence of a reproductive barrier. Comparison of the floral characters of *H. ravenii* and *X. gracile* indicated a high degree of morphological similarity (Matos 1979). In 1990, a single plant of *X. gracile* with 5 viable seeds was found in a *H. ravenii* population which produced highly sterile F1 hybrids indicating unsuccessful interspecific pollination. DNA content of three *X. gracile* accessions was also not found to be significantly different than a *H. ravenii* accession (Jackson et al. 1993).

This discrepancy in the taxonomic status of *X. gracile* and *H. ravenii,* wherein Jackson (1962, 1965, 1971) advocated for a separate species status for *H. ravenii* and other botanists (e.g., Cronquist 1971) and recent treatments of the genus *Xanthisma* based on phylogenetic studies (Morgan 1997a; Morgan and Hartman 2003; Hartman 2005; Morgan et al. 2009) do not recognize *H. ravenii* as separate from *X. gracile,* forms the premise for this study. We revisit the morphological distinctiveness and test for genetic differentiation of samples classified as each species to evaluate whether or not *H. ravenii* should be recognized. We consider the genotypic cluster criterion (GCC) (Mallet 1995) as the criteria for defining species. The GCC classifies groups of individuals based upon overall genetic similarity. It emphasizes genetic distinctiveness and defines species as: "morphologically and genetically identifiable clusters of individuals that can co-exist with other similar clusters with a few or no intermediates" (Mallet 1995). Herbarium specimens were utilized to measure morphological variation, and leaf samples from these specimens were used for genetic analyses of Amplified Fragment Length Polymorphisms (AFLP). If *H. ravenii* is distinct from *X. gracile* and this is due to reproductive isolation as claimed by Jackson (1965), then we expect that there would also be discontinuity in morphological characters and across the genome due to reduced gene flow leading to divergence in isolation.

MATERIALS AND METHODS

Plant Material and Morphological Measurements

A total of 201 herbarium specimens from Rancho Santa Ana Botanical Garden (RSA/POM, which includes RSA and Pomona College collections, n = 108), Arizona State University (ASU, n = 77), and Mississippi State University (MISSA, n = 16) were utilized in this study. The geographic areas covered

TABLE 1. Number of samples from U.S. states and Mexico assigned to *Haplopappus ravenii* and *Xanthisma gracile*.

Location	H. ravenii	X. gracile
Arizona, US	8	134
California, US	11	—
Colorado, US	—	4
New Mexico, US	—	16
Utah, US	5	—
Mexico	—	6

by these samples represent much of the range of *X. gracile* and *H. ravenii* in the southwestern United States (Table 1). The data set also included several specimens from the Sonora and Chihuahua regions of northern Mexico. The accession numbers and locations are listed in Appendix 1. Individuals were assigned to either *H. ravenii* or *X. gracile* based on morphological characters and geographic distributions mentioned in Jackson (1962), Jackson and Crovello (1971), and Morgan and Hartman (2003). If Jackson had annotated specimens as *H. ravenii*, then his identification was used without question, given that he was the foremost expert on this group. These included most of the San Bernardino populations, which agreed with the geographical range of *H. ravenii* (Jackson 1965). Samples from Arizona were treated as *H. ravenii* only if they were sampled from Yavapai County and matched the morphological description of Jackson (1962) in which he identified the type specimen and described the geographic distribution of this species in Arizona. All samples from Utah and California were considered to be *H. ravenii* because they fell in the geographic range described by Jackson (1962). All other samples were identified to species by following the key to the species level as described in Morgan and Hartman (2003). Using this approach, 24 specimens were identified as *H. ravenii* and 160 were identified as *X. gracile*.

Morphological variation was quantified in eight traits, which correspond to the characters used by Jackson and Crovello (1971) to differentiate *H. ravenii* from *X. gracile*. These traits include phyllary number, phyllary pubescence, leaf length, leaf width, number of teeth on leaf, pubescence of stem, achene length, and pappus length. Lack of mature seed sets in all the herbarium specimens limited the use of seed characters (achene and pappus length); thus these characters were not used in data analyses. The final dataset (n = 184) only included the individuals for which the first six morphological characters could consistently be measured. All measurements were made manually under an Olympus dissecting microscope using a miniscale (Bioquip, Rancho Dominguez, CA, USA). Measurements were made in triplicate on flowers, leaves and stems of each individual, and the average of these readings for each sample was used in data analyses. Mean values were used to account for maximum variation possible within a specimen. Phyllary number was estimated by

counting the rows of bracts. Phyllary pubescence was characterized as a binary character as appressed or stiff. Lengths were measured from point of attachment to the tip, and the longest leaves were measured at the bottom of the plant to rule out variation related to incomplete development. Leaf width was measured at the point of maximum width. Number of teeth on the leaf edge was counted. The pubescence of the stem was coded as a binary character as dense or sparse. Because leaf length and width can be correlated, we also considered a ratio of length to width for the leaves in the analyses. To avoid a potential bias based on wrong allocation to the groups, we also divided the specimens using just the phyllary pubescence as a differentiating factor, as suggested by Jackson and Crovello (1971). Based on this method, 96 specimens were identified as *H. ravenii* and 88 specimens as *X. gracile*.

DNA Extraction

To estimate genetic differentiation and genetic variation between the two species, 86 specimens from the 184 dataset were used (to reduce sample size bias). Fifteen specimens were identified as *H. ravenii* as described by Jackson (1962), whereas 71 were identified as *X. gracile*, and these represented different geographic locations. DNA was extracted from leaf tissue of herbarium specimens following a modified CTAB DNA extraction protocol (Doyle and Doyle 1987). After extraction, DNA was dissolved in 100 μl TE Buffer, treated with RNase A (10mg/ml, ABgene, Rochester, NY, USA) to remove any residual RNA, and run on a 1.5% agarose gel with ethidium bromide to check for quality.

AFLP Analysis

AFLP analysis was performed using a protocol modified from Vos et al. (1995) and incorporating the recommendations made by Trybush et al. (2006). Individual genomic DNA was digested in 30 μl reactions incubated at 37°C for one hr in a thermal cycler, followed immediately by ligation of the linkers. Restriction digest enzymes and reagents utilized per reaction were: 0.25 μl of *EcoRI* (20,000 U/ml, New England BioLabs, Ipswich, MA, USA), 0.5 μl of *MseI* (10,000 U/ml, New England Bio-Labs,), 3 μl 10X NEBuffer2, 0.15 μl of 100 μg/ml BSA, 5 μl of individually purified genomic DNA, and 21.1 μl of sterile water. Eco AFLP linkers were annealed in a thermal cycler by heating to 65°C for 10 min and cooled to room temperature using the following reagents: 10 μl of Eco Linker 1 (100 μM, 5′-CTC GTA GAC TGC CC) and 10 μl of Eco Linker 2 (100 μM, 5′-AAT TGG TAC GCA GTC TAC). Mse AFLP linkers were also prepared using the above procedure utilizing 10 μl of Mse Linker 1(100 μM, 5′-GAC GAT GAG TCC TGA G) and10

μl of Mse Linker 2 (100 μM, 5'-TAC TCA GGA CTC AT). Annealed linkers were stored frozen at −20°C until use.

Ligation of Eco and Mse linkers was conducted in 40 μl reactions, including 0.1 μl Eco and 1 μl Mse Linker (as annealed above), 0.15 μl T4 DNA Ligase enzyme and 4 μl 10X T4 DNA Ligase Reaction Buffer (New England BioLabs), 30 μl digested DNA, and 4.75 μl sterile water. Ligation reactions were incubated in a thermal cycler at 37°C for 4 hr followed by storage at −80°C to prevent degradation. Pre-selective amplifications were conducted in 10 μl reactions using 0.5 μl each Eco+A (10 μM, 5'-GAC TGC GTA CCA ATT CA) and Mse+C (10 μM, 5'-GAT GAG TCC TGA GTA AC) primers, 1.25 μl dNTPs (2 mM dATP, dCTP, dGTP, and dTTP; New England BioLabs), 2 μl 5X *GoTaq*™ FlexiBuffer (Promega Corp Madison, WI, USA), 1.25 μl MgCl₂ (Promega Corp.), 2.5 μl individually ligated DNA, 2.55 μl sterile water, and 0.1 μl *GoTaq*™ DNA polymerase (5u/μl, Promega Corp.). Pre-selective amplifications consisted of an initial denaturing step of 65°C for 5 min, 30-cycles of 30 sec at 94°C, 30 sec at 56°C, and one min at 72°C.

Pre-selective amplification products were individually diluted 1:20 with sterile water. Selective amplifications consisted of a single Mse primer with three fluorescent-labeled Eco primers per reaction. Selective amplification for all individuals was conducted in 10 μl volume consisting of 0.7 μl each Mse-CAG (5μM), Eco-ACT FAM, Eco-ACC NED and Eco-AGG VIC (1 μM) selective primers, 0.5 μl dNTPs (2 mM dATP, dCTP, dGTP, and dTTP; New England BioLabs), 2.5 μl LongAmp™ Buffer (New England Biolabs), 1 μl of diluted pre-selective amplification product, 2.7 μl sterile water, and 0.5 μl *LongAmp Taq*™ DNA polymerase (5u/μl, New England Biolabs). Selective amplifications consisted of an initial denaturing step of 95°C for 15 min, 13-cycles of 30 sec at 94°C, 1 min at 65°C, and 1 min at 72°C (reducing annealing temperature by 0.7°C/cycle), 25-cycles of 30 sec at 94°C, 30 sec at 55°C, and 1 min at 72°C, and finished with 10 min at 72°C. The selective amplification fragments were diluted 1:10 with distilled water, and 1 μl of the diluted fragments was transferred to a 96-well plate and allowed to air dry before being sent to Arizona State University for capillary electrophoresis with 0.3 μl LIZ-600 size standard (Life Technologies, Carlsbad, CA, USA) per sample. Peaks were visualized using GeneMarker® (Softgenetics, LLC, State College, PA, USA) and polymorphic bands were scored as present (1) or absent (0). Only bands in size ranges of 75–600 base pairs (bp) were used for all the primer combinations.

Data Analyses

For the four quantitative morphological characters, a t-test was run in R using the function *t.test* (R Development Core Team 2012) to determine if there were significant differences for each continuous trait among the samples assigned to each species. Additionally, because these species can be difficult to identify based solely on morphological traits, we also used clustering analysis to determine if natural breaks existed in the morphological data set (all six characters) that may correspond to different species. Because this analysis does not require a priori assignment of individuals to groups, we were able to evaluate whether there might have been inconsistency in how we assigned specimens to one species or the other in the t-tests. Additionally, because the clustering analysis can accommodate quantitative and qualitative characters, it allowed us to consider all of the characters, rather than focusing primarily on leaf characters (three of the four analyzed) in the t-tests. A non-hierarchical method for clustering, using *kmeans* function in R (R Development Core Team 2012) was conducted. The data were standardized by standard deviation such that each trait was equally weighted. K-means uses an a priori identified number of groups and utilizes an optimality criterion to fit the data within those groups. The sum of squares within each group is calculated and assigned to the predefined number of clusters to assess the best fit (Everitt 2005; Knaus 2008). As the number of groups increases, the sum of squares should decrease, and the optimal group number is identified by a sudden reduction in the sum of squares. To choose the most probable number of clusters represented by the data, an iterative method using the "calinski" criterion was conducted using the *cascadeKM* function in the vegan package of R software (R Development Core Team 2012). The "calinski" criterion is an analysis of variance statistic that compares the sum of squares among groups relative to the within group sum of squares. The value is plotted with respect to the cluster solutions, and the maximum value defines the number of groups, thereby providing the best *k means* solution. The null hypothesis is that k clusters are not significantly different and the larger the value of the calinski criterion, the better a group solution. We chose the "calinski" criterion from the other available criteria as it has been suggested to be able to provide the best solution after comparing it with 30 other such criteria (Milligan and Cooper 1985; R manual for Vegan library function cascadeKM, Oksanen et al. 2009). Principal components analysis was also performed using the *princomp* function in R (R Development Core Team 2012) to visualize groups as defined by the calinski criterion. Both of these analyses used all six morphological characters.

GenAlEx 6.0 (Peakall and Smouse 2001) was used to generate diversity statistics (i.e., percentage polymorphic loci, number of private bands, unbiased heterozygosity and Shannon's Index) and to conduct a hierarchical Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) to understand the partitioning of the genetic variation between samples assigned to each of the two species. Polymorphic

TABLE 2.   Mean and standard deviation (SD) of the morphological measurements and results of t-tests on four quantitative traits based on two different grouping schemes. A P-value of 0.05 was used to identify significantly different mean values.

Group	Character	Mean (SD) *ravenii*	Mean (SD) *gracile*	t	Degrees of freedom	P-value
Geography & annotation						
	Phyllary number	3(0.4)	3.38(0.67)	1.849	45.767	0.07093
	Leaf length	10.45(3.76)	10.13(4.10)	0.3792	31.762	0.7071
	Leaf width	0.9(0.26)	1.07(1.17)	1.826	158.744	0.06973
	Number of teeth	9.33(2.15)	11.97(3.01)	2.0065	38.132	0.05193
Phyllary pubescence						
	Phyllary number	3.3(0.63)	3.47 (0.67)	1.8295	178.114	0.06899
	Leaf length	9.47(3.74)	10.76(4.29)	2.1624	173.292	0.03196
	Leaf width	1.10(1.48)	0.99(0.38)	−0.73	108.604	0.467
	Number of teeth	11.09(3.16)	12.22(2.55)	2.6805	179.184	0.008038

bands that occur in a single group (i.e., species as defined by us) are referred to as private bands. To understand genetic relatedness among samples, a neighbor-joining (NJ) tree was constructed using the AFLP data binary matrix based on Nei-Li distances (Nei and Li 1979) in PAUP* v 4.0b10 (Swofford 2002), and a bootstrap (Felsenstein 1985) analysis was also conducted in PAUP* with 500 replicates to assess support of clusters. The consensus tree was visualized in iTOL (Letunic and Bork 2007, 2011).

RESULTS

Results from t-tests suggested that out of the four quantitative traits, none showed significant differences between the two groups when specimens were assigned based on geography and previous identification, but two characters were significantly different (Leaf length and Number of teeth) when the specimens were identified into groups using phyllary pubescence as a key character (Table 2). K-means analysis revealed a smooth curve without a natural break point, suggesting a single cluster (Fig. 1) as opposed to data sets in which a sharp elbow can be seen to differentiate the best cluster solution from others. This was further confirmed using the "calinski" criterion, which suggested a single cluster best fits the data (Table 3) because the highest within-group sum of squares value

is at one cluster. The PCA plot also lacks clear distinction of two groups that would correspond to X. *gracile* and *H. ravenii* (Fig. 2). In the PCA, the first two axes explain 56.1% variation, with number of teeth and leaf length showing the highest loadings on the first axis. Individuals from Yavapai County, where Jackson (1960b) had described the type specimen for *H. ravenii*, are not distinctive relative to other specimens in the PCA plot. No differences in groupings were identified when ratio of leaf length and width was used.

The AFLP primer combinations resulted in the generation of 856 polymorphic loci scored between 75 and 500 bp. The number of private bands detected in samples identified, as *X. gracile* was 400, with an average frequency of these bands was 0.47 (Table 4). Among samples assigned as *H. ravenii*, 22 bands were considered unique, although the average frequency of these bands was only 0.04. The percentage of polymorphic loci was 97.43% in *X. gracile* and 53.27% in *H. ravenii*. Shannon's Information Index (I) was 0.158 in *X. gracile* and 0.133 in *H. ravenii*. Nei's gene diversity values were lower in *H. ravenii* than *X. gracile* (0.075 and 0.079 respectively). AMOVA revealed that most of the variation was contained within groups (98%) rather than between groups (2%), although this was a significant value (P < 0.05; Table 5). We repeated the analyses by
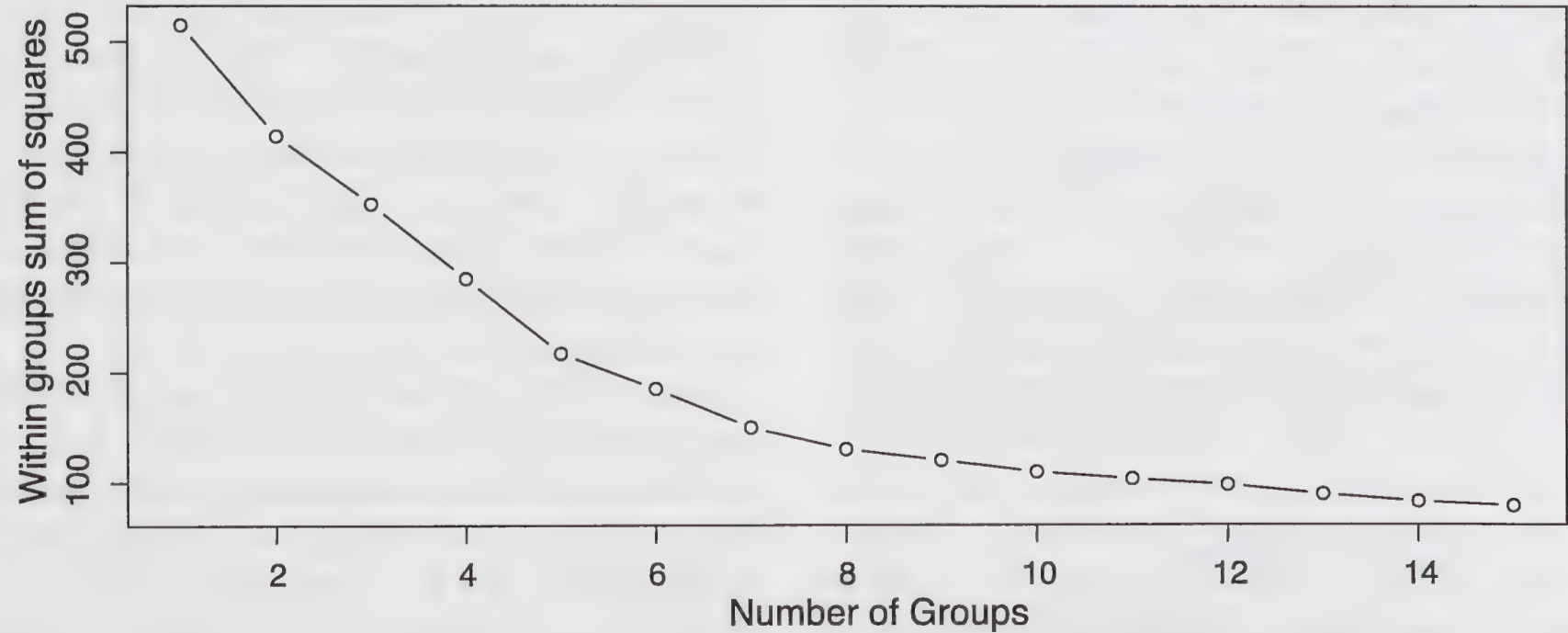
FIG. 1.   K-means clustering using six morphological characters depicting the number of groups based on within group sum of squares.

TABLE 3. Estimates of the calinski criterion utilizing the stopping rule of Calinski and Harabasz using the function cascadeKM.

Number of groups	1	2	3	4	5	6	7	8	9	10
Calinski criterion	Inf	42.329	39.556	36.889	35.402	34.734	35.104	35.006	34.688	33.685

dividing the data based on phyllary pubescence and did not change the results (Table 6). There was now only 1% difference accounted for between the groups in the AMOVA analysis (Table 7). The NJ tree did not reveal distinct clustering of the two species, although there were several strongly supported clusters in the tree (Fig. 3).

DISCUSSION

Jackson (1960b, 1962, 1965) conducted several studies to characterize the origin of the focal taxa and provided evidence that *H. ravenii* was an ancestral race from which *X. gracile* was derived, hence suggesting a progenitor-derivative relationship between the two (Crawford 2010). However, the recognition of *H. ravenii* as a unique species was questioned (e.g., Cronquist 1971) immediately after Jackson and Crovello (1971) proposed the independent species recognition, and it is not recognized in current taxonomic treatments of *X. gracile* (Morgan and Hartman 2003). The data collected for this study do not support recognition of *H. ravenii* at the species level either. Because we sampled similar geographic areas to those in Jackson and Crovello

(1971), and we used specimens annotated by Jackson, we expected to see distinct clusters corresponding to the individuals from Yavapai County, Arizona, Utah, and California, which were described as the range of *H. ravenii* by Jackson and Crovello (1971). However, no support for distinct clusters was found based on the morphological characters examined in this study. Jackson (1962) defined phyllary pubescence as one of the important distinguishing factors, with *X. gracile* exhibiting long appressed hair in comparison to *H. ravenii* with shorter, stiffer hairs. According to Jackson and Crovello (1971), phyllary pubescence is a diagnostic character that is always characterized as stiff when associated with *H. ravenii* and will effectively distinguish the two. We do not agree with this assessment as individuals assigned on the basis of geography and historical annotations to both groups in our study exhibited this character state. It is possible that our designated groups may have contained individuals that were assigned to the incorrect group, which would reduce the differences between these groups. However, the kmeans analysis, which seeks to find the structure in a data set in the absence of a priori assignment of individuals, also did not find any distinctive clusters that would indicate non-overlapping morphological characters. We conclude that the morphological differences identified by Jackson and Crovello (1971) and assigned to *H. ravenii* may be population-specific or plastic and are not continuous throughout the range of this taxon. Reduced pollen fertility (on average 6.9%) in the F1 hybrids from *H. ravenii* and *X. gracile* suggested a barrier to gene exchange leading to reproductive isolation and hence speciation (Jackson 1962; Jackson et al. 1993). However, these studies did not comment on the possibility of the presence of supernumerary chromosomes, which are abundantly described in the two taxa (Jackson 1960a, b; Raven et al. 1960; Li and Jackson 1961), contributing to the reduced fertility. Pritchard (1968) provided evidence for an exponential decrease in pollen fertility confounded with an increase in the number of supernumerary chromosomes, making this a possibility for these species as well.

Because morphology can be misleading of underlying genetic diversity or evolutionary history, we also tested for genetic differentiation among the sampled individuals. If the species are reproductively isolated, then they should be expected to exhibit genetic divergence due to reduced gene flow. According to the GCC (Mallet 1995), this would provide evidence of separate species. There is little evidence in our genetic data set to support that *H. ravenii* is genetically distinct from *X. gracile*. No
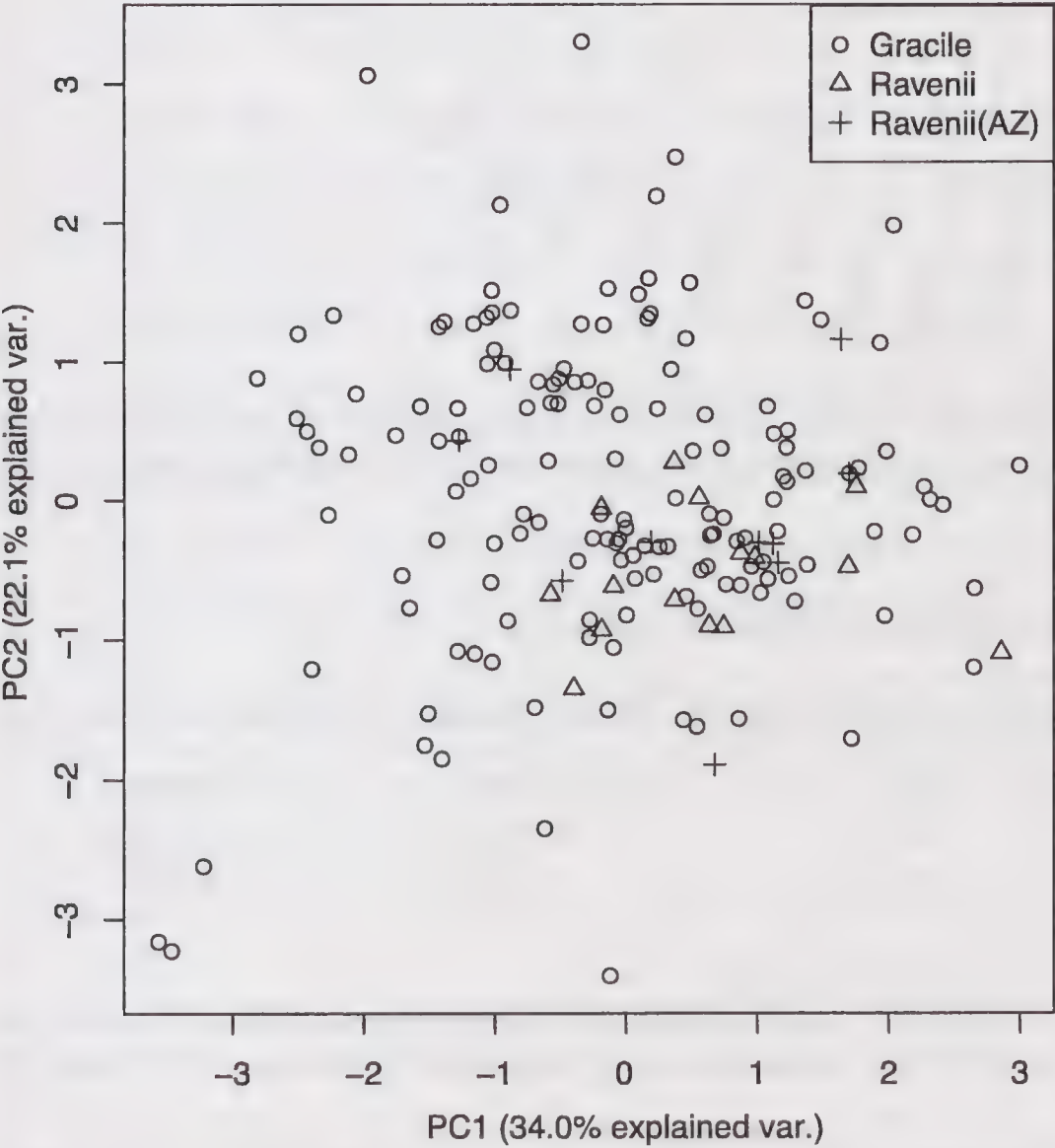
FIG. 2. Scatter plot of principal components #1 and #2 (PC1 and PC2) based on six morphological characters. Combined these two components explain 56.1% of the morphological variation. Number of teeth on leaf and leaf length had the highest loadings on the first axis.
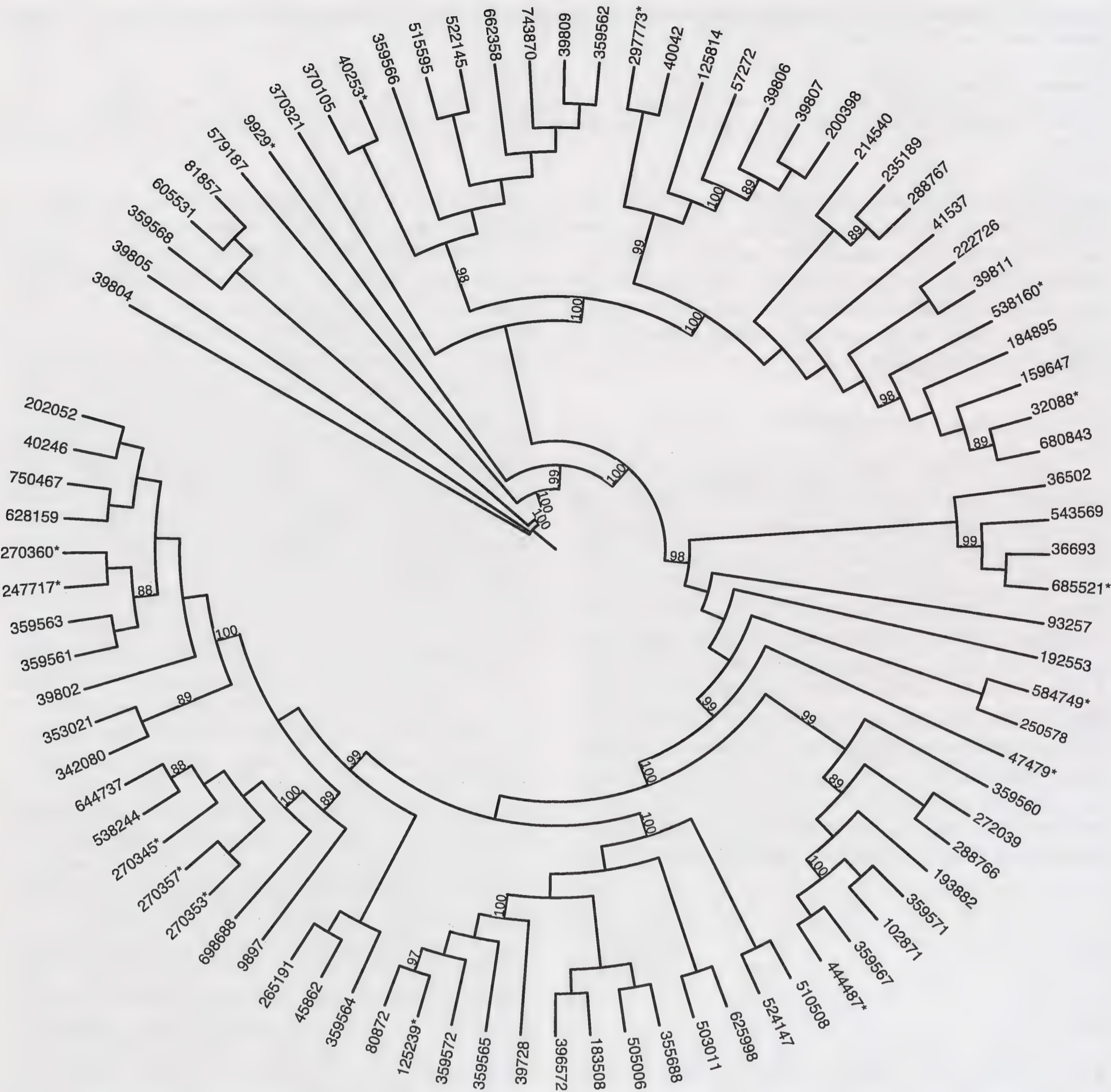
FIG. 3. Neighbor joining tree based on the AFLP presence/absence binary matrix depicting genetic relatedness among samples of *X. gracile* and *H. ravenii*. The numbers on branches represent bootstrap values >80% and the tip labels are the accession numbers (* denotes the *H. ravenii* specimens).

TABLE 4. Estimates of genetic diversity between *Xanthisma gracile* and *Haplopappus ravenii*.

Species	Sample size	Percentage polymorphic loci	Number of private alleles	Shannon index	Nei's gene diversity
X. gracile	71	97.43	400	0.158	0.079
H. ravenii	15	53.27	22	0.133	0.075
Mean	13.33	75.35	211	0.146	0.077

TABLE 5. Analysis of Molecular Variance (AMOVA) based on AFLP variation among samples assigned to *Xanthisma gracile* or *Haplopappus ravenii*. Note: P-value estimate based on 9999 permutations. df = degrees of freedom, SS = sum of squares, and MS = mean squared deviations.

Source	df	SS	MS	Estimated variance	% of total variation	Phi	P-value
Among groups	1	81.245	81.245	0.899	2%		
Within groups	84	4858.488	57.839	57.859	98%	$\phi_{ST} = 0.015$	<0.05
Total	85	4939.733		58.738	100%		

COMING NEXT IN *MADROÑO* 63(4)

Jim Thorne, "California's Historic Legacy for Landscape Change, the Wieslander Vegetation Type Maps."

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The Wieslander Vegetation Type Map (VTM) project was the first systematic survey of California's forests and woodlands. Conducted by the U.S. Forest Service over a 12-year period beginning in 1926, the survey crews covered nearly half the state, surveyed about 16,000 vegetation plots, and hand-drew the patterns of vegetation onto topographic maps. They also took over 3,000 photos, and collected thousands of herbarium specimens (now housed in the University and Jepson Herbaria at UC Berkeley, and available online through the Consortium of California Herbaria).

For the first time, the digital rendition of the VTMs is presented in summary form in the upcoming issue of *Madroño* 63(4).

Photograph of a VTM project crew

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TABLE 6.   Estimates of genetic diversity between *Xanthisma gracile* and *Haplopappus ravenii* after dividing them based on phyllary pubescence.

Species	Sample size	Percentage polymorphic loci	Number of private alleles	Shannon index	Nei's gene diversity
X. gracile	41	86.33	106	0.154	0.078
H. ravenii	45	87.62	117	0.153	0.079
Mean	43	86.97	111.5	0.153	0.078

distinct clusters were identified in the NJ analysis (Fig. 3), and AMOVA indicated a much greater amount of genetic diversity within (98%) than among groups (2%). Although the among-group variance was significant, we found no genetic markers that were unique and in high frequency in either group. The significant variation among groups is perhaps due to the extensive amount of variation observed in this dataset (i.e., nearly every individual exhibited a unique genotype) and the great differences in sample sizes of the two groups. For recently derived species where there has not been sufficient time to accumulate genetic differences, variation in overall diversity can indicate progenitor-derivative relationships (Crawford 2010). We did find that *H. ravenii* samples exhibited less diversity than *X. gracile* as measured by the percent of polymorphic loci, Nei's gene diversity or Shannon's index (Table 4), but this is heavily influenced by differences in sample size. Furthermore, Jackson (1962, 1965) suggested that *H. ravenii* was the more ancestral species; thus it is expected to contain a higher amount of diversity compared to *X. gracile* (Crawford 2010).

Species complexes exhibiting phenotypic, genetic, and/or cytological variation can be problematic for a stable taxonomy. Several similar studies have been conducted in other plant taxa, which address questions raised in this study. For example, studies of the wild potato complex (Alvarez et al. 2008; Fajardo et al. 2008), which consist of morphologically similar species, resulted in the authors reducing the number of species due to lack of diagnostic morphological traits or genetic markers. In an analysis of two species of *Senecio* L., also based on morphological and genetic data, Pelser and Houchin (2004), found overlap across characters leading to the suggestion that the varietal rank was the best taxonomic solution for this complex.

In light of the lack of support for sufficient divergence across both morphology and genetic data in this study, the only non-overlapping difference between the two taxa appears to be the difference in chromosome number. Given that we did not

characterize cytotypes in this study, it is difficult to reconcile the apparent reproductive isolation between cytotypes (Jackson 1965) with the lack of morphological or genetic divergence we observed. Additional studies of reproductive isolation between these cytotypes with varied geographic distributions, as well as characterization of supernumerary chromosomes, would be very helpful to evaluate whether these were local effects observed by Jackson (1962).

CONCLUSIONS

Both the morphological data, which were based on the same characters used by Jackson and Crovello (1971), and the genetic data fail to provide support that *H. ravenii* is distinct. The presence of different chromosome numbers along with the highly sterile hybrids with intermediacy in characters separating the two suggests that they are cytotypes of *X. gracile*. Although the genetic differentiation between the two taxa is statistically significant in an AMOVA, a larger proportion of genetic variation was detected among individuals in the two groups. Thus, we do not consider this sufficient to warrant their recognition as separate species at this time.

LITERATURE CITED

ALVAREZ, N. M. B., I. E. PERALTA, A. SALAS, AND D. M. SPOONER. 2008. A morphological study of species boundaries of the wild potato *Solanum brevicaule* complex: replicated field trials in Peru. Plant Systematics and Evolution 274:37–45.

BARRETT, C. F. AND J. V. FREUDENSTEIN. 2011. An integrative approach to delimiting species in a rare but widespread mycoheterotrophic orchid. Molecular Ecology 20:2771–2786.

CALINSKI, T. S. AND J. HARABASZ. 1974. A dendrite method for cluster analysis. Communications in Statistics 3:1–27.

CRAWFORD, D. J. 2010. Progenitor-derivative species pairs and plant speciation. Taxon, 1413–1423.

CRONQUIST, A. 1971. Note on *Haplopappus gracilis* and *Haplopappus ravenii*. Brittonia 23:292.

CRUZ-BARRAZA, J. A., J. L. CARBALLO, A. ROCHA-OLIVARES, H. EHRLICH, AND M. HOG. 2012. Integra-

TABLE 7.   Analysis of Molecular Variance (AMOVA) based on AFLP variation among samples assigned to *Xanthisma gracile* or *Haplopappus ravenii* after dividing them based on phyllary pubescence. Note: P-value estimate based on 9999 permutations. df = degrees of freedom, SS = sum of squares, and MS = mean squared deviations.

Source	df	SS	MS	Estimated variance	% of total variation	Phi	P-value
Among groups	1	76.554	76.554	0.435	1		
Within groups	84	4863.178	57.895	57.895	99	$\phi_{ST} = 0.007$	<0.05
Total	85	4939.733		58.33	100		

tive taxonomy and molecular phylogeny of genus *Aplysina* (Demospongiae: Verongida) from Mexican Pacific. PLoS ONE 7:e42049.

DOYLE, J. J. AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.

EVERITT, B. 2005. An R and S-plus companion to multivariate analysis. Springer, London, UK.

EXCOFFIER, L., P. E. SMOUSE, AND J. M. QUATTRO. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

FAJARDO, D., R. CASTILLO, A. SALAS, AND D. M. SPOONER. 2008. A morphometric study of species boundaries of the wild potato *Solanum* series *Conicibaccata*: a replicated field trial in Andean Peru. Systematic Botany 33:183–192.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

HALL, H. M. 1928. The genus *Haplopappus*: a phylogenetic study in the Compositae. Carnegie Institute Washington Publications 389.

HARTMAN, R. L. 2005. *Xanthisma* (Asteraceae). Pp. 383–393 *in* Flora North America Editorial Committee (eds.). Flora of North America North of México, Vol. 20: Magnoliophyta: Asteridae (in part): Asteraceae, part 2. Oxford University Press, New York, NY.

JACKSON, R. C. 1957. New low chromosome number for plants. Science 126:1115.

———. 1960a. Documented chromosome numbers of plants. Madroño 15:220.

———. 1960b. Supernumerary chromosomes in *Haplopappus gracilis*. Evolution 74:135.

———. 1962. Interspecific hybridization in *Haplopappus* and its bearing on chromosome evolution in the Blepharodon section. American Journal of Botany 49:119–132.

———. 1965. A cytogenetic study of a three paired race of *Haplopappus gracilis*. American Journal of Botany 52:946–953.

———. 1971. Note on *Haplopappus gracilis* and *Haplopappus ravenii*—a reply. Brittonia 23(4):446.

——— AND T. J. CROVELLO. 1971. A comparison of numerical and biosystematic studies in *Haplopappus*. Brittonia 23(1):54–70.

———, C. F. CRANE, J. S. JOHNSTON, J. R. ELLISON, AND H. J. PRICE. 1993. Aneuploidy, structural chromosome changes, and DNA amounts in the annual taxa of the *Haplopappus spinulosus* complex. Chromosome Research 1993, 1:53–61.

KEARNEY, T. H. AND R. H. PEEBLES. 1951. Arizona Flora. University of California Press, Berkeley, CA.

KNAUS, B. J. 2008. Morphometric architecture of the most taxon- rich species in the US Flora: *Astragalus lentiginosus* (Fabaceae) American Journal of Botany 97:1816–1826.

LETUNIC, I. AND P. BORK. 2007. Interactive Tree of Life (iTOL): an online tool for phylogenetic display and annotation. Bioinformatics 23:127–128.

———. 2011. Interactive Tree of Life v2: online annotation and display of phylogenetic trees made easy. Nucleic Acids Research 39:W475–W478.

LEVIN, D. A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, New York.

LI, N. AND R. C. JACKSON. 1961. Supernumerary chromosomes in *Haplopappus spinulosus* ssp. *cotula*. American Journal of Botany 48:419–426.

LÖVE, D. 1960. Nomenclature notes on Mt. Washington plants. Taxon 17:89.

MALLET, J. 1995. A species definition for the modern synthesis. Trends in Ecology & Evolution 10:294–299.

MILLIGAN, G. W. AND M. C. COOPER. 1985. An examination of procedures for determining the number of clusters in a data set. Psychometrika 50:159–179.

MORGAN, D. R. 1997a. Reticulate evolution in *Machaeranthera* (Asteraceae). Systematic Botany, 22:599–615.

——— AND R. L. HARTMAN. 2003. A synopsis of *Machaeranthera* (Asteraceae: Astereae), with recognition of segregate genera. Sida 20:1387–1416.

———, R-L. KORN, AND S. L. MUGLESTON. 2009. Insights into reticulate evolution in *Machaeranthera* (Asteraceae: Astereae): 5S ribosomal RNA spacer variation, estimating support for incongruence, and constructing reticulate phylogenies. American Journal of Botany, 96:920–932.

NEI, M. AND W. H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 76:5269–5273.

OKSANEN, J., F. G. BLANCHET, R. KINDT, P. LEGENDRE, R. G. O'HARA, G. L. SIMPSON, P. SOLYMOS, M. HENRY, H. WAGNER, AND J. OKSANEN. 2010. Vegan: community ecology package. R package, version 1.17-0. Website: http://CRAN.Rproject.org/package=vegan

PADIAL J. M., A. MIRALLES, I. DE LA RIVA, AND M. VENCES. 2010. The integrative future of taxonomy. Frontiers in Zoology 7:16.

PEAKALL, R. AND P. E. SMOUSE. 2006. GENALEX 6: Genetic analyses in excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

PELSER, P. B. AND R. HOUCHIN. 2004. Taxonomic studies on Senecio aquaticus (Asteraceae). A recommendation for the taxonomic status of *Aquaticus* and *Barbareifolius*. Botanical Journal of the Linnaean Society 145:489–498.

PRITCHARD, E. 1968. A cytogenetic study of supernumerary chromosomes in *Haplopappus gracilis*. Canadian Journal of Cytology and Genetics 10:928–936.

R DEVELOPMENT CORE TEAM. 2012 R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

RAMSEY, J. AND D. W. SCHEMSKE. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annual Review of Ecology and Systematics 29:467–501.

RAVEN, P. H., O. T. SOLBRIG, D. W. KYHOS, AND P. SNOW. 1960. Chromosome numbers in Compositae. I. Astereae. American Journal of Botany 47:124–132.

SCHLICK-STEINER, B. C., F. M. STEINER, B. SEIFERT, C. STAUFFER, E. CHRISTIAN, AND R. H. CROZIER. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. Annual Review of Entomology. 55:421–438.

SWOFFORD, D. L. 2002. PAUP* Phylogenetic Analysis Using Parsimony and other methods, Version 4. Sinauer Associates, Sunderland, Massachusetts.

TANAKA, R. 1967. A comparative karyotype analysis in Haplopappus gracilis (2n = 4) and H. ravenii (2n = 8). Cytologia 32:542–552.

TRYBUSH, S., S. HANLEY, K. H. CHO, Š. JAHODOVÁ, M. GRIMMER, I. EMELIANOV, C. BAYON, AND A. KARP.

2006. Getting the most out of fluorescent amplified fragment length polymorphism. Canadian Journal of Botany 84:1347–1354.

VOS, P., R. HOGERS, M. BLEEKER, M. REIJANS, T. VAN DE LEE, M. HORNES, A. FRITERS, J. POT, J. PALEMAN, M. KUIPER, AND M. ZABEAU. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23:4407–4414.

WHITE, M. J. D. 1968. Models of speciation. Science 159:1065–1070.

APPENDIX 1. List of accessions from Rancho Santa Ana Botanical Garden and Pomona College (RSA/POM), Arizona State University Herbarium (ASU), and Mississippi State University Herbarium (MISSA). Herbarium Specimens: Accession Numbers, Counties, States, Geographic Coordinates and specimen labels.

Accession No.	County	State	Latitude	Longitude	Specimen labels	Herbarium
12202	Coconino	AZ	35.1866	−111.618	A1	ASU
12203	Coconino	AZ	35.1981	−111.651	A2	ASU
12204	Gila	AZ	33.74	−110.93	A3	ASU
12205	Navajo	AZ	34.2542	−110.029	A4	ASU
12206	Maricopa	AZ	33.9686	−112.729	A5	ASU
12208	Pima	AZ	31.8078	−110.594	A6	ASU
12209	Coconino	AZ	35.8333	−112.083	A7	ASU
12210	Pinal	AZ	33.3722	−111.201	A8	ASU
12213	Gila	AZ	33.6485	−111.114	A9	ASU
12214	Gila	AZ	33.6008	−110.517	A10	ASU
12215	Maricopa	AZ	33.8642	−111.467	A11	ASU
12217	Maricopa	AZ	33.5323	−111.369	A12	ASU
12218	Cochise	AZ	31.4481	−109.928	A13	ASU
12219	Maricopa	AZ	33.7916	−111.467	A14	ASU
12221	Gila	AZ	34.3152	−111.016	A15	ASU
12224	Yavapai	AZ	34.46	−112.43	A16	ASU
12228	Maricopa	AZ	33.5454	−111.452	A17	ASU
12229	Pima	AZ	31.7639	−110.749	A18	ASU
12250	Coconino	AZ	35.1866	−111.618	A19	ASU
12323	Pima	AZ	31.5747	−111.332	A20	ASU
12330	Coconino	AZ	35.1981	−111.651	A21	ASU
12331	Gila	AZ	34.2308	−111.324	A22	ASU
12332	Santa Cruz	AZ	31.4092	−111.127	A23	ASU
12333	Pima	AZ	32.5	−110.921	A24	ASU
12337	Santa Cruz	AZ	31.5394	−110.756	A25	ASU
12340	Cochise	AZ	31.9341	−109.117	A26	ASU
12342	Navajo	AZ	33.7906	−109.988	A27	ASU
12348	Gila	AZ	34.3086	−111.343	A28	ASU
12355	Graham	AZ	32.67	−109.88	A29	ASU
12379	Gila	AZ	33.6478	−111.114	A30	ASU
12380	Navajo	AZ	34.4314	−110.593	A31	ASU
12381	Pinal	AZ	33.2794	−111.157	A32	ASU
12382	Yavapai	AZ	34.2017	−112.763	A33	ASU
12383	Yavapai	AZ	34.5833	−112.6	A34	ASU
12386	Gila	AZ	33.6891	−111.241	A35	ASU
12389	Gila	AZ	34.1014	−110.963	A36	ASU
15917	Cochise	AZ	31.4269	−110.455	A37	ASU
15935	Maricopa	AZ	33.8984	−111.823	A38	ASU
15965	Graham	AZ	33.1786	−109.863	A39	ASU
15966	Greenlee	AZ	33.4	−109.333	A40	ASU
15971	Yavapai	AZ	34.4247	−113.241	A41	ASU
15972	Santa Cruz	AZ	31.5148	−110.727	A42	ASU
16024	Gila	AZ	33.2822	−110.821	A43	ASU
16025	Yavapai	AZ	34.5833	−112.6	A44	ASU
20059	Santa Cruz	AZ	31.4092	−111.085	A45	ASU
39433	Apache	AZ	NA	NA	A4	ASU
78098	Gila	AZ	33.6941	−110.587	A47	ASU
78354	Santa Cruz	AZ	31.4611	−111.331	A48	ASU
78362	Navajo	AZ	34.0482	−110.222	A49	ASU
81357	Pinal	AZ	33.28	−111.17	A50	ASU
83050	Maricopa	AZ	33.5975	−111.205	A51	ASU
83899	Maricopa	AZ	33.9686	−112.729	A52	ASU
85188	La Paz	AZ	33.8224	−113.384	A53	ASU
88116	Graham	AZ	32.65	−109.82	A54	ASU
88380	Coconino	AZ	34.8262	−111.76	A55	ASU
101360	Maricopa	AZ	33.8642	−111.467	A56	ASU
113414	Mohave	AZ	34.9567	−113.678	A57	ASU
146889	Apache	AZ	34.5341	−109.307	A58	ASU
160847	Gila	AZ	34.2859	−111.664	A59	ASU
160881	Cochise	AZ	31.8825	−109.203	A60	ASU
167286	La Paz	AZ	33.91	−113.63	A61	ASU

APPENDIX 1.   CONTINUED.

Accession No.	County	State	Latitude	Longitude	Specimen labels	Herbarium
184895	Maricopa	AZ	33.5333	−111.333	A62	ASU
190241	Pinal	AZ	NA	NA	A63	ASU
200251	Mohave	AZ	36.7849	−113.232	A64	ASU
211369	Pima	AZ	32.4303	−110.705	A65	ASU
229082	Graham	AZ	32.9251	−110.167	A66	ASU
238214	Mohave	AZ	36.8983	−112.918	A67	ASU
240927	Pinal	AZ	32.9765	−110.777	A68	ASU
245401	Cochise	AZ	31.7197	−110.163	A69	ASU
246109	Yavapai	AZ	34.659	−111.749	A70	ASU
263703	Maricopa	AZ	33.982	−111.71	A71	ASU
263830	Santa Cruz	AZ	31.6184	−110.496	A72	ASU
264708	Maricopa	AZ	33.97	−111.861	A73	ASU
264788	Maricopa	AZ	33.9662	−111.863	A74	ASU
266649	Yavapai	AZ	34.495	−112.545	A75	ASU
268506	Pima	AZ	31.7769	−110.722	A76	ASU
270622	Gila	AZ	33.72517	−110.99	A77	ASU
LC01	Maricopa	AZ	33.26482	−111.17558	X_01	MISSA
LC03	Pinal	AZ	35.1562	−111.68711	X_03	MISSA
LC04	Mohave	AZ	34.8498	−111.82837	X_04	MISSA
LC05	Pima	AZ	34.81525	−111.90518	X_05	MISSA
LC06	Graham	AZ	34.70784	−112.1489	X_06	MISSA
LC07	Mohave	AZ	34.67791	−112.187	X_07	MISSA
LC08	Coconino	AZ	34.51982	−112.45229	X_08	MISSA
LC09	Cochise	AZ	34.35759	−112.38261	X_09	MISSA
LC10	Yavapai	AZ	34.37462	−112.25768	X_10	MISSA
LC11	Maricopa	AZ	31.7841	−110.6965	X_11	MISSA
LC12	Santa Cruz	AZ	31.78249	−110.74254	X_12	MISSA
LC13	Maricopa	AZ	31.67921	−110.65564	X_13	MISSA
LC14	Maricopa	AZ	31.60082	−110.57849	X_14	MISSA
LC15	Yavapai	AZ	31.54105	−110.51155	X_15	MISSA
LC16	Pima	AZ	31.54361	−110.33031	X_16	MISSA
LC17	Gila	AZ	31.96148	−110.34653	X_17	MISSA
444487	San Bernardino	CA	NA	NA	R1	RSA/POM
644737	Coconino	AZ	NA	NA	R10	RSA/POM
190478	Coconino	AZ	NA	NA	R100	RSA/POM
270357	San Bernardino	CA	NA	NA	R101	RSA/POM
288765	Coconino	AZ	NA	NA	R102	RSA/POM
359570	Coronado	AZ	NA	NA	R103	RSA/POM
359573	Otero	NM	NA	NA	R104	RSA/POM
359631	NM	NM	NA	NA	R105	RSA/POM
488278	Santa Cruz	AZ	NA	NA	R106	RSA/POM
498868	San Antonio, lower CA	CA	NA	NA	R107	RSA/POM
575452	Hidalgo	NM	NA	NA	R108	RSA/POM
39806	Chihuahua	Mexico	NA	NA	R11	RSA/POM
125239	San Bernardino	CA	NA	NA	R12	RSA/POM
685521	Yavapai	AZ	NA	NA	R13	RSA/POM
359565	showlow springerville hwy	AZ	NA	NA	R14	RSA/POM
359563	Cochise	AZ	NA	NA	R15	RSA/POM
359571	Archuleta	CO	NA	NA	R16	RSA/POM
285676	Coconino	AZ	NA	NA	R17	RSA/POM
579187	Santa Cruz	AZ	NA	NA	R18	RSA/POM
247797	San Bernardino	CA	NA	NA	R19	RSA/POM
270360	San Bernardino	CA	NA	NA	R2	RSA/POM
763349	San Bernardino	CA	NA	NA	R20	RSA/POM
750467	Cochise	AZ	NA	NA	R21	RSA/POM
743870	Pinal	AZ	NA	NA	R22	RSA/POM
41537	Pima	AZ	NA	NA	R23	RSA/POM
39804	Pima/Santa Cruz	AZ	NA	NA	R24	RSA/POM
39805	Pima/Santa Cruz	AZ	NA	NA	R25	RSA/POM
193882	Cochise	AZ	NA	−NA	R26	RSA/POM
359567	Blue River	AZ	NA	NA	R27	RSA/POM
168770	Sonora	Mexico	NA	NA	R28	RSA/POM
81857	Santa Cruz	AZ	NA	NA	R29	RSA/POM

APPENDIX 1. CONTINUED.

Accession No.	County	State	Latitude	Longitude	Specimen labels	Herbarium
270345	San Bernardino	CA	NA	NA	R3	RSA/POM
270353	San Bernardino	CA	NA	NA	R30	RSA/POM
200398	Gila Bend	AZ	NA	NA	R31	RSA/POM
625998	NM-AZ state line	NM_AZ	NA	NA	R32	RSA/POM
359572	Dona Ana	NM	NA	NA	R33	RSA/POM
45862	near Santa Fe	NM	NA	NA	R34	RSA/POM
39572	Sierra	NM	NA	NA	R35	RSA/POM
40042	Grant	AZ	NA	NA	R36	RSA/POM
39802	Grant	AZ	NA	NA	R37	RSA/POM
39728	Dona Ana	NM	NA	NA	R38	RSA/POM
39809	Pinos Altos	NM	NA	NA	R39	RSA/POM
297773	NA	NA	NA	NA	R4	RSA/POM
265191	Deming Luna	NM	NA	NA	R40	RSA/POM
662358	Pima	AZ	NA	NA	R41	RSA/POM
272039	Cochise	AZ	NA	NA	R42	RSA/POM
342080	Coconino	AZ	NA	NA	R43	RSA/POM
510508	Yavapai	AZ	NA	NA	R44	RSA/POM
515595	Pima	AZ	NA	NA	R45	RSA/POM
522145	Pima	AZ	NA	NA	R46	RSA/POM
524147	Yavapai Co.	AZ	NA	NA	R47	RSA/POM
543569	Maricopa	AZ	NA	NA	R48	RSA/POM
222726	Nye	NV	NA	NA	R49	RSA/POM
102871	Beaver Dam	AZ	NA	NA	R5	RSA/POM
32088	Washington	UT	NA	NA	R50	RSA/POM
40253	Kanab	UT	NA	NA	R51	RSA/POM
538160	Washington	UT	NA	NA	R52	RSA/POM
125814	Dona Ana	NM	NA	NA	R53	RSA/POM
605531	Santa Cruz	AZ	NA	NA	R54	RSA/POM
39725	Dona Ana	NM	NA	NA	R55	RSA/POM
355688	Sierra	NM	NA	NA	R56	RSA/POM
80872	Dona Ana	NM	NA	NA	R57	RSA/POM
359566	Santa Cruz	AZ	NA	NA	R58	RSA/POM
370105	Santa Cruz	AZ	NA	NA	R59	RSA/POM
57272	Durango	NM	NA	NA	R6	RSA/POM
359561	Cochise	AZ	NA	NA	R60	RSA/POM
628159	Cochise	AZ	NA	NA	R61	RSA/POM
359568	Sheldon	AZ	NA	NA	R62	RSA/POM
39807	Chihuahua	Mexico	NA	NA	R63	RSA/POM
495325	Sonora	Mexico	NA	NA	R64	RSA/POM
584749	San Bernardino	CA	NA	NA	R65	RSA/POM
299176	San Bernardino	CA	NA	NA	R66	RSA/POM
47479	San Bernardino	CA	NA	NA	R67	RSA/POM
698688	Coconino	AZ	NA	NA	R68	RSA/POM
680843	Mohave	AZ	NA	NA	R69	RSA/POM
36502	La Plata	CO	NA	NA	R7	RSA/POM
505006	NA	NA	NA	NA	R70	RSA/POM
359564	Coyote Mountain	AZ	NA	NA	R71	RSA/POM
370321	Santa Cruz	AZ	NA	NA	R72	RSA/POM
353021	Coconino	AZ	NA	NA	R73	RSA/POM
268286	Mohave	AZ	NA	NA	R74	RSA/POM
39811	Pima	AZ	NA	NA	R75	RSA/POM
202052	Coconino	AZ	NA	NA	R76	RSA/POM
214540	Pima	AZ	NA	NA	R77	RSA/POM
250578	AZ	AZ	NA	NA	R78	RSA/POM
359562	Santa Cruz	AZ	NA	NA	R79	RSA/POM
192553	Mohave	AZ	CNA	NA	R8	RSA/POM
9897	Coconino	AZ	NA	NA	R80	RSA/POM
288766	Cochise	AZ	NA	NA	R81	RSA/POM
655901	Sonora	Mexico	LaNA	Longitude	R82	RSA/POM
235189	Pima	AZ	NA	NA	R83	RSA/POM
159647	Hurricane rd nw AZ	AZ	NA	NA	R84	RSA/POM
36693	Mesa	CO	NA	NA	R85	RSA/POM
40246	Coconino	AZ	NA	NA	R86	RSA/POM

APPENDIX 1.   CONTINUED.

Accession No.	County	State	Latitude	Longitude	Specimen labels	Herbarium
183508	Void	Void	NA	NA	R87	RSA/POM
503011	El Salto	NA	NA	NA	R88	RSA/POM
119099	Sonora	Mexico	NA	NA	R89	RSA/POM
359560	Cochise	AZ	NA	NA	R9	RSA/POM
288767	Pima	AZ	NA	NA	R90	RSA/POM
9929	Yavapai	AZ	NA	NA	R91	RSA/POM
93257	Mohave	AZ	NA	NA	R92	RSA/POM
39735	Yavapai	AZ	NA	NA	R93	RSA/POM
538244	Coconino	AZ	NA	NA	R94	RSA/POM
39808	Pima/Santa Cruz	AZ	NA	NA	R95	RSA/POM
39810	Cochise	AZ	NA	NA	R96	RSA/POM
40252	Springdale	UT	NA	NA	R97	RSA/POM
102869	Yavapai/Maricopa	AZ	NA	NA	R98	RSA/POM
117951	Mt Meadows	UT	NA	NA	R99	RSA/POM

POST-WINDSTORM RADIAL GROWTH OF *PICEA SITCHENSIS* AND *PSEUDOTSUGA MENZIESII*

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ABSTRACT

This paper presents a case study examining the radial-growth history of Sitka spruce (*Picea sitchensis*) and Douglas-fir (*Pseudotsuga menziesii*) trees following known and projected high-wind events along the central Oregon coast. Using tree-ring measurements collected from wind-traumatized trees of both species, we identified periods exhibiting negative (−) and positive (+) radial growth corresponding to canopy breakage (−) and increased light levels following canopy gap formation (+). Periods of negative and positive growth were then statistically and qualitatively compared across species and against a period of historically documented windstorms (AD 1895–2003) and a 230-year tree-ring derived windstorm record (AD 1775–2003). Our results revealed: 1) both Sitka spruce and Douglas-fir exhibit radial-growth anomalies corresponding to high-magnitude windstorms during the period of historical period, and 2) Sitka spruce is more sensitive and susceptible to wind-induced canopy trauma and exhibits an earlier onset of post-windstorm recovery than Douglas-fir over a >230-year period. These results are consistent with the combined effects of topography and coastal proximity, each species' habitat requirements, and their physiological tolerances to wind stress.

Key Words: Dendroecology, Douglas-fir, mid-latitude cyclones, Pacific Northwest, Sitka spruce, wind disturbance, windstorms.

The role of wind on forest processes is well-established in the ecological literature (Coutts and Grace 1995; Everham and Brokaw 1996; Peterson 2000), yet few studies provide the temporal and physical evidence needed to characterized windstorm disturbance regimes over periods encompassing decades to centuries (Knapp and Hadley 2012). The lack of historical perspective contrasts sharply with the abundance of tree-ring research that quantify the frequency and severity of historical fire regimes (e.g., Arabas et al. 2006), insect outbreaks (e.g., Speer et al. 2001), and extreme weather events such as hurricanes (e.g., Busby et al. 2009), drought (e.g., Stahle et al. 2000), and ice storms (Lafon and Speer 2002). Here we contribute toward a dendroecological understanding of wind disturbance by presenting a 230-year history of Sitka spruce (*Picea sitchensis* [Bong.] Carr.) and coast Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *menziesii*) tree-growth responses to high-wind events (HWEs) along the central Oregon coast.

Derived from mid-latitude cyclones and northeastern Pacific typhoon remnants (Mass and Dotson 2010; Read 2013), Pacific Northwest (PNW) HWEs commonly exceed hurricane-velocity winds (118 kph) with a landfall frequency equal to or exceeding those of Atlantic and Gulf Coast hurricanes (Mass and Dotson 2010). Consistent with the >1000 km radius of extratropical cyclones (Patoux et al. 2009), PNW HWEs are regional in their extent affecting coastal areas from northern California to British Columbia (Mass and Dotson 2010) concomitant with the overlapping geographic range of Sitka spruce (39°30′–57°30′N) and coast Douglas-fir (35°–51°N) (Earle 2011). PNW windstorms include 49 well-documented HWEs along the Oregon and Washington coast between 1880 and 2008 (Read 2013). Among these events, 1880, 1934, 1940, 1945, 1950, 1951, 1962, 1963, 1981, 1993, 1995, 1999, 2006, and 2007 were major storm years (MSYs) associated with high-magnitude, high-severity weather conditions over a large (500–1000 km) area (Mass and Dotson 2010; NOAA 2011; Read 2013). Earlier MSYs identified from tree-ring data collected at seven sites along the Oregon coast include: 1746, 1774, 1797, 1798, 1799, 1800, 1832, 1861, 1863, 1869, and 1878 (Knapp and Hadley 2012).

Similar to the mid-latitude cyclones that frequent the forest ecosystems of western South America and Europe (Rebertus et al. 1997; Schelhass et al. 2003), PNW windstorms have profound ecological consequences spanning a broad range of geographic scales from low-frequency, large-scale (>500 km^2) forest blowdown (>2.3 million m^3) (Ruth and Yoder 1953; Lynott and Cramer 1966; Ruth and Harris 1979) to high-frequency, small-scale canopy changes resulting from branch loss and windsnap of upper bole sections (e.g., Spies and Franklin 1989; Lertzman et al. 1996; Kramer et al. 2001). Accordingly, PNW windstorms are now recognized as a primary

disturbance agent governing the region's forest structure and succession (Green et al. 1992; Franklin and Halpern 2000) and forest resource inventories (Harcombe et al. 2004; Wilson 2004).

An important ecological consequence of HWEs is the alteration of forest structure (White 1979; Lertzman et al. 1996). These structural changes include tree mortality from windthrow and stem breakage, canopy loss of limbs and foliage, and the concomitant formation of canopy gaps (Ott and Juday 2002; Lutz and Halpern 2006). These processes subsequently lead to increased spacing and lower density among surviving, canopy-dominant trees (Franklin et al. 1981), a corresponding increase in air flow (Gray et al. 2009), and increased susceptibility to subsequent canopy gap expansion (Foster and Reiners 1986). Windsnap and windthrow also increase the number and density of snags (Greene et al. 1992; Gray et al. 2012) and the accumulation of coarse woody debris (CWD) including potential nurse logs (Harmon et al. 1986; Harmon and Franklin 1989). Windthrow further contributes to a hummocky (pit and mound) micro topography and the exposure of mineral soil horizons excavated by tree-tipped root wads (Schaetzl et al. 1989).

Canopy gaps are recognized for their role in forest succession in closed-canopy forests (White 1979) including Sitka spruce- (Harcombe 1986) and Douglas-fir- (Gray et al. 2012) dominated forests of the PNW. Representing light-enriched environments, canopy gaps can trigger ecological release among light-constrained understory plants (Van Pelt and Franklin 2000; Kennedy and Quinn 2001; but see Halpern and Lutz 2013) including seedlings and saplings (Van Pelt and Franklin 1999). The influx of CWD from limb and stem breakage, and small-scale changes in microclimate contribute to greater environmental (White 1979; Gray et al. 2009) and understory species diversity, and community succession (Pabst and Spies 1999; McDonald 2013). Canopy gap size and orientation, and local understory composition are important factors determining post-disturbance successional pathways (Harcombe 1986; Spies et al. 1990; Kane et al. 2011).

Notwithstanding these and other effects of HWEs on forest structure and succession, our understanding of how windstorms influence long-term rates and patterns of tree growth remains vague. Here we address two questions regarding post-windstorm the tree growth: Do the radial-growth patterns of Sitka spruce and coast Douglas-fir differ in response to HWEs within the same study site, and if so, what might these differences tell us about the relative sensitivity, susceptibility, and recovery of these species to HWEs? Based on these outcomes we discuss how each species' autecology, landscape location, and habitat conditions influence their sensitivity and susceptibility to wind disturbance.

METHODS

Study Area

Our study site is located in the adjacent, west-east trending Cummins Creek and Gwynn Creek drainage basins ca. one km south of Cape Perpetua (44.3°N, 124.1°W) on Oregon's central coast (Fig. 1). We selected this site because it facilitated our post-windstorm growth comparison in two ways. First, Cape Perpetua's western extension and central Oregon coast location make it highly susceptible to the landfall and high winds of mid-latitude cyclones (Knapp and Hadley 2012; Read 2013). Second, the study area includes one of the few remaining old-growth (>350 years) forest remnants along the central Oregon coast that includes adjacent stands dominated by Sitka spruce or Douglas-fir.

Pacific Northwest conifers in general, and Sitka spruce and coast Douglas-fir in particular, exhibit some of the highest growth rates and greatest biomass of any trees in the world (Waring and Franklin 1979). These species represent the largest (Douglas-fir) and second largest (Sitka spruce) members of the Pinaceae commonly exceeding 70 m in height and three m in diameter (Harris 1990; Hermann and Lavender 1990). Both species exhibit rapid growth during their first 100 years when they approach or exceed 50 m height (Farr and Harris 1979; Hermann and Lavender 1990). Important ecological differences between these species include the disturbance conditions required for their regeneration success—wind disturbance (Sitka spruce) and fire (Douglas-fir) are the single most-important factors ensuring the long-term persistence (>1000 years) of these seral species.

Sitka spruce dominates the lower reaches of both drainages of our study site characterized by low-angled terrain associated with floodplain, river terraces, debris-flow deposits, and bar-and-swale topography with wet depressions and small discontinuous stream channels. These sites have a high percent shrub cover (McDonald 2013), high understory diversity (Pabst and Spies 1998), an abundance of shade-tolerant hardwood bigleaf maple (*Acer macrophyllum* Pursh), and high volumes of coarse woody debris (Wimberly and Spies 2001).

Douglas-fir replaces Sitka spruce as the dominant tree species in adjacent up-valley locations on moderate-to-steep (ca. 10–25°) hillslopes. These sites support a less diverse understory consistent with their lower environmental diversity and disturbance frequency (Pabst and Spies 1998). These drier, upstream sites experienced fires at least once during 19th and 20th centuries (Wimberly and Spies 2001) with the last severe subregional fire occurring in 1849 (Morris 1934; Impara 1997). Co-dominant tree species in the study area include two shade-tolerant, late-successional species, western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) and western red cedar (*Thuja plicata* Donn ex D. Don). Red alder (*Alnus rubra* Bong.) is a
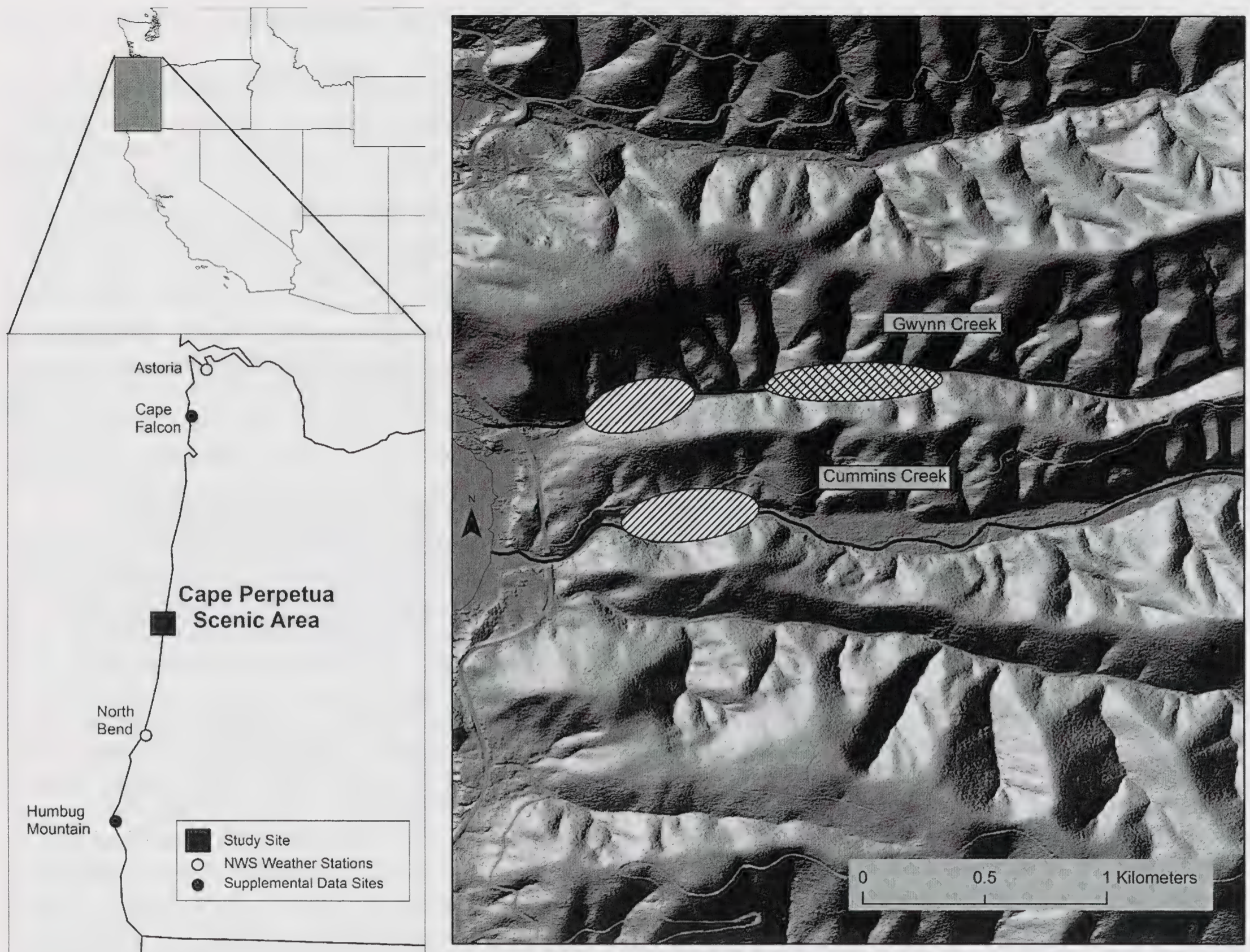
FIG. 1. Location of the Cape Perpetua study site, weather stations, reference sites, and the Cummins Creek and Gwynn Creek drainages. Locations of sampled trees are indicated by a line pattern (Sitka spruce) and cross pattern (Douglas-fir).

common forest component in areas experiencing recent disturbance (Wimberly and Spies 2001).

Canopy gaps derived from crown breakage and individual (ca. 50 m^2) and multiple treefall (ca. ≥500 m^2) events are common throughout the study area concomitant with its old-growth forest structure (Franklin et al. 1981; Lertzman et al. 1996). Similar to other seral coniferous species, Sitka spruce and Douglas-fir experience self-thinning during early stages of stand development (Oliver 1981) but can experience periods of accelerated growth if triggered by higher light levels in the lower canopy (Winter et al. 2002; Stan and Daniels 2010; Gray et al. 2012). Complex canopy structures are common among older trees (Ishii and McDowell 2002; Van Pelt and Sillett 2008) including the rapid growth of lower limbs as they expand laterally into gap openings and vertically to replace windsnapped or dead portions of the main tree stem (Fig. 2) (Hadley and Knapp 2011). The sustained long-term growth rates and longevity of both species may also be related to their capacity to develop epicormic branches (Winter et al. 2002; Van Pelt and Sillett 2008) in response to canopy disturbance.

Consistent with its marine, west-coast location, Cape Perpetua experiences high (ca. 171–230 cm/yr) winter-season precipitation (November–March) with a narrow mean annual temperature range of ca. 11.2°C (10.6°–11.7°). Freezing temperatures occur <20 days/year and total annual snow fall is < 2.5 cm. Mean monthly summer temperatures vary between ca. 10–24.5°C. Saturated air and fog drip are common during the low-precipitation months of June–September (data ranges represent values for Newport and Tidewater, Oregon) (NCDC 2011; WRCC 2011).

Field Sampling

Our field sampling consisted of collecting two 18 to 71 cm cores using 61 to 81 cm increment borers at ≥1.4 m height from live, large-diameter (>1 m), wind-snapped, canopy-emergent, old-growth Sitka spruce (23 trees; 47 cores) and Douglas-fir (22 trees; 46 cores) identified during field traverses of the lower reaches of Cummins and Gwynn Creeks. Each wind-snapped tree was visually confirmed by two observers using two or more of the following features: a missing upper bole, a missing crown apex, apical
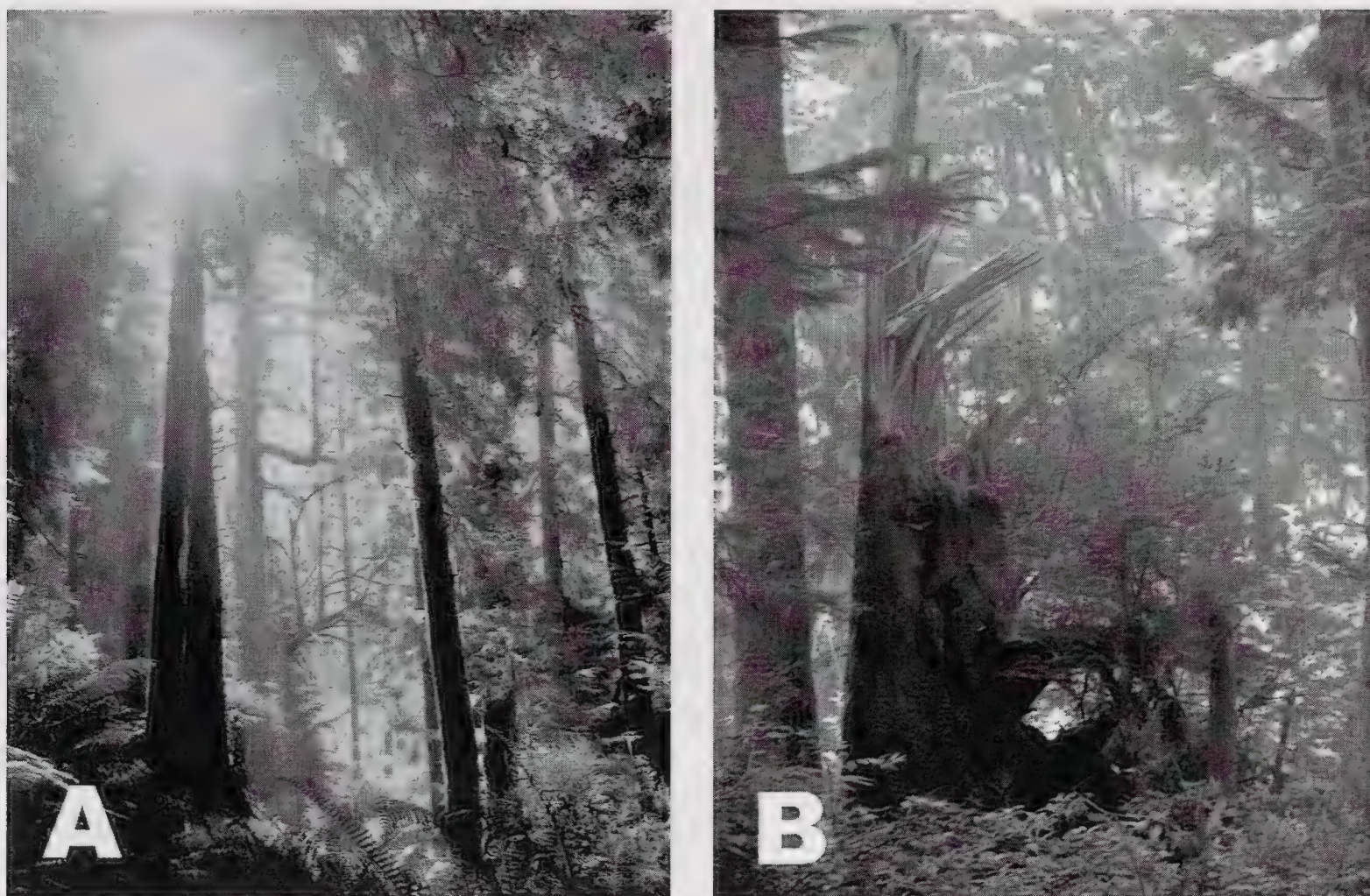
FIG. 2.   Examples of wind-induced canopy disturbance at the Cape Perpetua: (A) an upturned limb ("muscle arm") leading to apical dominance following an earlier HWE, and (B) a wind-snapped Sitka spruce bole resulting in canopy gap formation.

crown replacement, or a splintered bole (Fig. 2). Diameter at breast height (dbh) for Sitka spruce (median: 240 cm; range: 156–377 cm) and Douglas-fir (median: 222 cm; range: 171–273 cm) were measured for each sampled tree. Sitka spruce canopy heights (median: 51.6 m; range: 37.8–82.2 m) and Douglas-fir canopy heights (median: 52.4 m; range: 36.9–62.2 m) were opportunistically measured using an Impulse laser rangefinder.

Tree-Ring Analysis

A primary objective of our tree-ring analysis was to maximize the detection of HWEs while minimizing the potential of false positive, Type I Errors. Accordingly, we approached this objective using a twofold approach. This entailed the use of raw ring-width measurements to: 1) maximize the identification of growth anomalies and 2) more precisely assess the frequency, ratio, and coincidence of growth anomalies identified in individual trees over the 1775–2003 period of reliability (≥5 cores). We then used standardized ring-width indices for our more conservative, inferential population comparisons of long-term tree growth (1775–2003) and the frequency and timing of growth anomalies relative to the historical HWE record (1895–3003).

We determined historical windstorm dates by identifying Sitka spruce and Douglas-fir tree-ring growth anomalies representing years of suppressed (–) or accelerated (+) tree growth caused by wind-induced canopy trauma or the creation of new canopy openings. Years recording growth anomalies were then compared to historically documented HWEs (Read 2013) and HWE years (1895–2003) identified from monthly wind-speed records recorded at Astoria, Oregon and North Head, Washington

(Hadley and Knapp 2011; Knapp and Hadley 2012). An additional 27 HWE years were interpreted from accounts of storm-related shipwrecks along the Oregon and Washington coast between 1849 and 1991 (Gibbs 1993). We then compared our tree-ring results to major windstorm years (MSYs) identified in a 300-year tree-ring record representing seven Oregon coast locations (Knapp and Hadley 2012).

Individual Tree Analysis

Each tree core used in our tree-ring analyses was prepared for ring counts and measurements using standard drying, mounting (Speer 2010), and sanding procedures (Orvis and Grissino-Mayer 2002). Ring counts and visual crossdating were made using variable-powered, stereo-microscopes. Initial cross-dating was completed following the list method (Yamaguchi 1991). Tree rings in all visually cross-dated cores were then measured to 0.01 mm using a VELMEX measuring bench yielding raw ring-width (non-standardized) values used in our individual tree analysis.

We identified HWE-related growth releases and growth suppressions in each core using a growth-boundary approach that compared 5-year ring-width segments with the adjacent 5-year segments similar to a moving window over the entirety of the sample (Veblen et al. 1991). Segments exhibiting >50% increase in mean ring width relative to the previous 5-year segment were identified as growth releases; segments identified with a 50% decrease in mean ring width were identified as growth suppressions. Both growth anomaly thresholds were defined using an empirical "best match" approach that optimized the correlation between historically documented HWEs and growth anomalies (Hadley and Knapp 2011). We

thus defined "best match" as the % change threshold that minimized the number of false positives (detection of a HWE in the absence of a documented event) and the number of false negatives (non-detection of HWE noted in the historical record). Years corresponding to matches, false positives and false negatives were not estimated for our individual tree assessment because of the absence of windstorm documentation over most of the 1775–1895 period.

We used periods of suppression and release to reconfirm our visual cross-dating accuracy; only successfully crossdated cores were used for our final analyses. We then compared the number and percentage of growth-anomalies for both species for the period having a sample depth of $\geq$5 cores (1775–2003). The 2003 ending date ensured a five-year comparison period required by our method.

Population Analysis

Each visually crossdated core used in our population analysis was statistically checked to ensure crossdating accuracy using the program COFECHA (ver. 6.06) (Holmes 1983; Grissino-Mayer 2001). Tree cores used in our population analysis were then standardized using the program ARSTAN ver. 6.05 (Cook and Holmes 1986) applying a 30-year cubic spline following experimentation and visual assessment of standardization procedures (Hadley and Knapp 2011). This procedure removed age-related growth trends while retaining 99% of the high-frequency growth variation over a 9.3-year period (Hadley and Knapp 2011). We ensured the reliability of our population analysis by restricting our comparisons to a period near-constant sample depth for both species during the period of reliable historical windstorm documentation (1895–2003).

Growth anomalies were identified by noting all $\geq$50% negative (suppressions) or positive (releases) deviations in index values in each tree-ring series (Hadley and Knapp 2011). We then combined the standardized individual tree series for each species using ARSTAN to construct our Sitka spruce and Douglas-fir chronologies representing the study site (Fig. 3A). The standardized tree-ring (chronology) data were then used to examine population differences in radial growth between Sitka spruce and Douglas-fir by comparing their interseries correlations and mean sensitivities.

Growth anomalies identified using our standardized tree-ring data were compared to the historically documented windstorm record (1895–2003) by assigning a match when tree-growth anomalies present in >15% of our cores occurred within two years of a preceding HWE. Years lacking growth anomalies during the two years following a documented HWE year were identified as a false negative. Years having tree-growth anomalies but lacking a documented HWE during the preceding two years were classified as false positives. The efficacy of this procedure was previously demonstrated in two earlier comparisons

that matched growth anomaly records with maximum peak wind gusts (all known hurricane-force winds) recorded at Astoria and North Bend, Oregon (Hadley and Knapp 2011) and North Head, Washington (Knapp and Hadley 2012). These results showed that the number of HWEs identified in >15% of the sampled cores were significantly greater (P < 0.001, two-tailed Mann-Whitney test) than those identified in <15% of the cores.

We tested the hypothesis that our standardized ring-width values for Sitka spruce and Douglas-fir during the 1775–2003 period were dissimilar using the Shapiro-Wilk normality and Wilcoxon signed-rank tests. Segments of each chronology were then examined using the expressed population signal (EPS) to provide additional measure of sample depth variability within and between our 1775–2003 chronologies. Following Speer (2010), we used an EPS value of 0.85 to identify segments of the crossdated chronologies to ensure our results were not dominated by individual trees. We used Gleichläufigkeit values (G-scores) to provide a measure of congruent positive or negative growth between two tree-ring populations in the same year allowing a direct comparison of growth anomalies between our two chronologies for the same year (Speer 2010). G-score and EPS calculations followed those presented in Speer (2010) derived from Wigley et al. (1984) and Briffa and Jones (1990).

We used Spearman rank correlations to assess the temporal relationship between individual and combined species growth anomalies and HWEs. Bivariate event analysis (e.g., Berg et al. 2006; Bigler et al. 2007) was deemed inappropriate based on our abbreviated record (109-years), near-constant sample depths, diminished value of annual probability-estimates, and the non-stationary (continuous) nature of HWEs relative to other climate phenomena (e.g., drought) and related disturbance agents (e.g., fire and insect outbreaks). We addressed the possibility of age-related sensitivities to HWEs using a post-hoc examination of standardized growth variance among the oldest trees sampled at seven coastal sites in Oregon (Knapp and Hadley 2012). This assessment revealed growth during the 20th century (0.010) was slightly less than the 19th century (0.011) but greater than the 18th century (0.08). These results suggest long-term growth rates of old-growth Sitka spruce and coast Douglas-fir vary independent of tree age and their growth sensitivities to HWEs and post-HWE recovery persist for >300 years (cf. Winter et al. 2002; Van Pelt and Sillett 2008).

Tree Growth and Climate

Radial growth of old-growth Sitka spruce and coast Douglas-fir are poorly associated with monthly climatic variables (Hadley and Knapp 2011; Knapp and Hadley, 2012) and appear unrelated to historically documented climate events and other growth-altering factors including insect defoliation, disease,
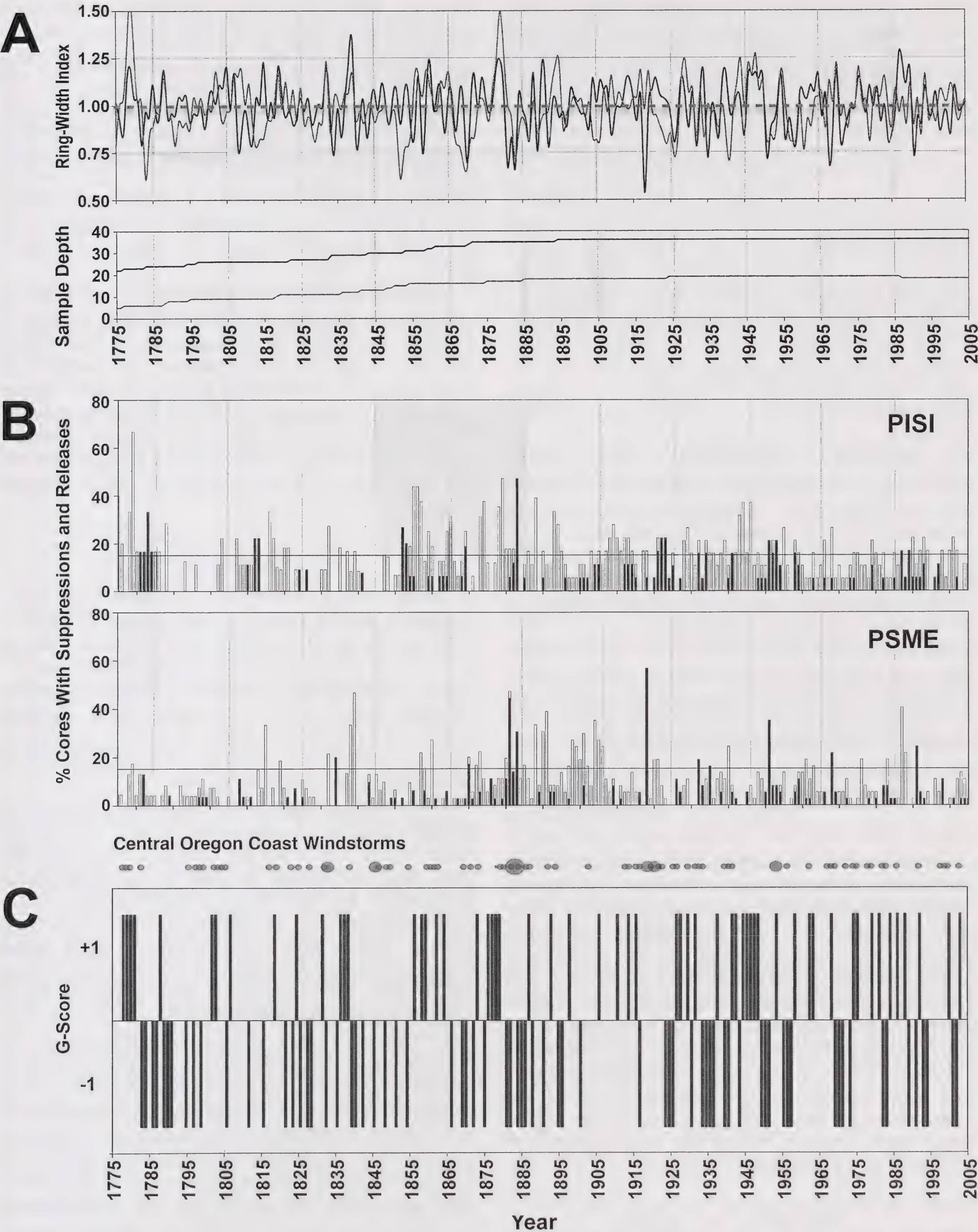
FIG. 3. Cape Perpetua tree-ring data for Sitka spruce and Douglas-fir for the 1775–2003 period. (A) Master ring-width chronologies (standardized ring-width series) and sample depth for Sitka spruce (black line) and Douglas-fir (gray line). (B) Percent of Sitka Spruce (PISI) and Douglas-fir (PSME) trees exhibiting annual growth suppressions (>50% growth reduction; black bars) and releases (>50% growth increase; gray bars) derived from crossdated, raw ring-width values; black horizontal lines identify 15% anomaly thresholds. (C) Gleichläufigkeit values (G-scores) showing years of congruent positive or negative growth derived from standardized ring-width series; Windstorms bubbles represent the documented occurrence of central Oregon Coast windstorms (Source: Gibbs 1993; NOAA 2011; Knapp and Hadley 2012; Read 2013). Sample depth for all figures shown in Fig. 3A. Vertical lines added to facilitate visual comparisons.

TABLE 1. Tree-ring statistics for windsnapped Sitka spruce and Douglas-fir trees comprising the entirety of the Cape Perpetua chronologies (1660-2003) where the sample size is ≥5. Data analyzed in the text represent individual trees for the period between 1775 and 2003 and tree populations for the period of documented windstorms between 1895 and 2003.

	Sitka spruce	Douglas-fir
Number of trees	16	19
Number of dated series	20	37
Master series	339	407
Total rings in all series	4039	9778
Total dated rings checked	4027	9762
Series intercorrelation	0.333	0.543
Average mean sensitivity	0.256	0.224
Segments with potential problems	69	31
Mean length of series	201.9	264.3

fire, and snow/ice storms (Hadley and Knapp 2011). Accordingly, we assumed growth variations occurring in old-growth, windsnapped trees at Cape Perpetua were caused by HWEs but acknowledge that other factors including Swiss needle-cast defoliation may induce growth variation in some younger, non-windsnapped trees (e.g., Black et al. 2010). We tested this assumption for our Cape Perpetua tree-ring data by comparing the standardized index values for both species to mean monthly temperature, mean monthly precipitation, and Palmer Drought Severity Index (PDSI) derived for Oregon Climate Division 1 (NOAA 2010) using linear regression models.

RESULTS

Twenty-seven Sitka spruce and nine Douglas-fir cores were unsuccessfully crossdated because of multiple periods of multiple missing tree-rings. These cores represented the most traumatized trees in our samples and were excluded from our analyses to ensure conservative results and lower probability of Type I Errors (i.e., assignment of false positive years). Discounting these cores, we based our results on 20 crossdated tree-ring series representing 16 Sitka spruce trees (3389 rings) and 37 crossdated tree-ring series representing 19 Douglas-fir trees (7618 rings) over the length of our chronologies (1775–2003) when the core sample size was ≥5 for both species (Fig. 3A, Table 1).

Individual Tree Analysis

Fifteen percent of our non-standardized Sitka spruce tree-ring measurements between 1775–2003 included growth anomalies. Growth suppressions accounted for 28% and growth releases 72% of the Sitka spruce anomalies. Sitka spruce anomalies were noted in 94% of the years comprising the 1895–2003 historical period. MSYs were identified in 46% of the Sitka spruce cores between 1775 and 2003, and in

53% of our cores during the 109-year historical period.

Approximately 10% of all Douglas-fir rings recorded a growth anomaly between 1775 and 2003. Growth suppression accounted for 31% and growth releases 69% of the Douglas-fir anomalies. Eighty-six percent of the Douglas-fir rings recorded growth anomalies during the 1895–2003 period yielding a return interval of 1.2 years. We identified MSYs in 19% of the Douglas-fir cores between 1775 and 2003, and 22% during the 109-year historical period.

Adjusted for sample-size differences, Sitka spruce suppressions and releases exceed the total number of number of Douglas-fir anomalies by ca. 2.25 times. Based on the recorded number of Douglas-fir anomalies and the adjusted number of Sitka spruce anomalies, we estimated the HWE return intervals at Cape Perpetua during the 1775–2003 period as 2.9 years for Sitka spruce, 10.4 years for Douglas-fir, and 5.9 years for the combined-species return interval (Fig. 3B).

Population Analysis

Mean growth sensitivity—a measure of high-frequency growth variation—was greater (0.256) for Sitka spruce than Douglas-fir (0.224) (Table 1) across the entirety of our standardized chronologies (1660–2003) consistent with our lower crossdating success of Sitka spruce cores. This high-growth variation among Sitka spruce is similar to those reported by Taylor (1990) and (Knapp and Hadley 2012) elsewhere along the Oregon coast.

Series intercorrelations for the entire length of the chronologies with a sample size of n ≥ 5 (0.333 for Sitka spruce and 0.543 for Douglas-fir) were lower than those reported for six other coastal chronologies constructed for the Oregon coast (range: 0.397–0.554) (Knapp and Hadley 2012). Statistical comparison of Sitka spruce and Douglas-fir index values revealed a significant difference between chronology values (P = <0.001; Shapiro-Wilk normality test / Wilcoxon signed-rank test).

EPS results (Table 2) show Douglas-fir exceeded 0.83 for each of the 13 periods beginning in 1660 versus four periods for Sitka spruce. G-scores—the proportion (or percentage) of years in which the two tree species ring widths increased or decreased together from prior year—revealed 96 positive, 133 negative, and two neutral-growth years for the 1775–2003 period (Fig. 3C). Both species exhibited positive growth for 57 years (25%) of the 229-year study period, negative growth for 68 (29%) years during the study period, and different growth trends for 108 (46%) of the study period.

Our tree population results for the 1895–2003 historical period revealed a total of 640 growth anomalies with Sitka spruce exhibiting 225% more anomalies than Douglas-fir despite a smaller sample size (20 vs. 37) of crossdated cores and fewer

TABLE 2. Expressed population analysis (EPS) results for Douglas-fir and Sitka spruce comprising the entirety of the Cape Perpetua chronologies (1660–2003) where the sample size is ≥5. EPS values exceeding 0.85 noted in bold; values between >0.83 and <0.85 noted in italics. Running correlation (r-bar) for 25-year periods exceeding the interseries correlation for each species noted in bold.

Year	r-bar	sdev	EPS	Ave # cores
Sitka spruce				
1730	0.26	0.27	0.43	2
1755	0.02	0.27	0.06	3
1780	0.09	0.25	0.35	6
1805	0.21	0.17	0.70	9
1830	0.13	0.17	0.63	11
1855	0.19	0.20	0.78	15
1880	0.23	0.22	*0.84*	17
1905	0.22	0.21	*0.83*	18
1930	0.26	0.19	**0.87**	19
1955	0.24	0.21	**0.86**	19
1980	0.13	0.19	0.74	19
Douglas-fir				
1660	0.38	0.19	*0.84*	8
1685	0.31	0.19	*0.83*	11
1710	0.27	0.19	*0.84*	14
1735	0.25	0.18	**0.85**	17
1760	0.28	0.19	**0.89**	20
1785	0.27	0.20	**0.89**	23
1810	0.24	0.22	**0.89**	26
1835	0.31	0.20	**0.93**	28
1860	0.30	0.16	**0.93**	32
1885	0.25	0.18	**0.92**	35
1910	0.26	0.18	**0.93**	36
1935	0.33	0.16	**0.95**	36
1960	0.30	0.15	**0.94**	36

measured rings (1885 vs. 4033). Adjusted for the difference in tree-ring sample size, Sitka spruce experienced 544% more growth anomalies than Douglas-fir consistent with the former's higher mean sensitivity, lower interseries correlation, and lower EPS values for all periods (Tables 1 and 2). The combined species exhibited growth anomalies in each of the 80-documented HWE years with Sitka spruce having a 100% match and a 79% match for Douglas-fir. Results for the historical period also displayed sustained periods of suppressed radial growth among Sitka spruce and a greater coincidence of growth anomalies among Douglas-fir following high-magnitude storm years and storm clusters in 1926, 1931–1932, 1940, 1958, 1991, and 1999 (Fig. 4).

The occurrence of false-positive years (FPYs) and false-negative years (FNYs) were consistent with the relative sensitivity of Sitka spruce and Douglas-fir. Sitka spruce yielded a larger number of FPYs (11) and number of false positive anomalies (44) than Douglas fir (6 FPS; 11 anomalies). The 1:14 ratio of FPYs identified in our 1895–2003 Sitka spruce chronology (109 years) and the number of documented HWE years (80) equates to ca. 8 additional HWEs over the historical period. Adjusting our HWE detection number to include the sum of actual

and projected FPYs (99) over the historical period approximates the near-annual (1.1 HWE/year) HWE frequency suggested by Hadley and Knapp (2011), the State of Oregon Natural Hazard Mitigation Plan (2015), and our individual tree results. Douglas-fir lacked growth anomalies (FNYs) in 14-documented HWE years.

The research implications of FPYs identified in our Sitka spruce chronology are consistent with the conservative results of our population analysis. These implications include: 1) our reliance on insufficient regional and site-specific windstorm data, 2) an underestimation of high-frequency, short-term growth variations inherent to standardized tree-ring series applied to different populations (tree species) and sample sizes, and 3) imprecise lag-time adjustments between physical events (HWEs) and biological responses (tree growth). We addressed the latter two issues by applying a common standardization procedure over the historical period having the most similar sample depth and examining how 3- to 4-year lag times might decrease the number of false positives. In the latter case, we found 97% of the Sitka spruce growth anomalies occurred within three years of a documented HWE; all growth anomalies occurred within four years of a documented HWE. Ninety-nine percent of all Douglas-fir growth anomalies occurred within three years following a documented HWE with all growth anomalies occurring within four years of a documented HWE. We deemed the merit of these adjustments unjustified based on the near-annual regional occurrence of HWEs, a decrease in dating precision, and inaccurate results resulting from the temporal overlap of longer response windows.

Our regression models between mean radial-growth indices for windsnapped Sitka spruce and Douglas-fir (dependent variable) and monthly climatic data from Oregon Climate Division 1 (independent variables) (NCDC 2010) revealed little correlation between climate and tree growth. Only April mean temperature exhibited a significant correlation with Sitka spruce annual radial growth ($r = 0.298$, $P = 0.002$); we found no detectable correlation ($P < 0.05$ between Douglas-fir and climate.

DISCUSSION

Windstorm Sensitivity

Our results—lower crossdating success, lower interseries correlation, higher mean sensitivity, significantly different tree-ring index populations, higher frequency of raw and standardized growth anomalies, and greater period variability indicated by EPS and G-scores—suggest individual trees and sample population of Sitka spruce are more sensitive and/or susceptible to high-frequency wind disturbance than Douglas-fir. The higher coincidence between the Sitka spruce growth anomalies at Cape
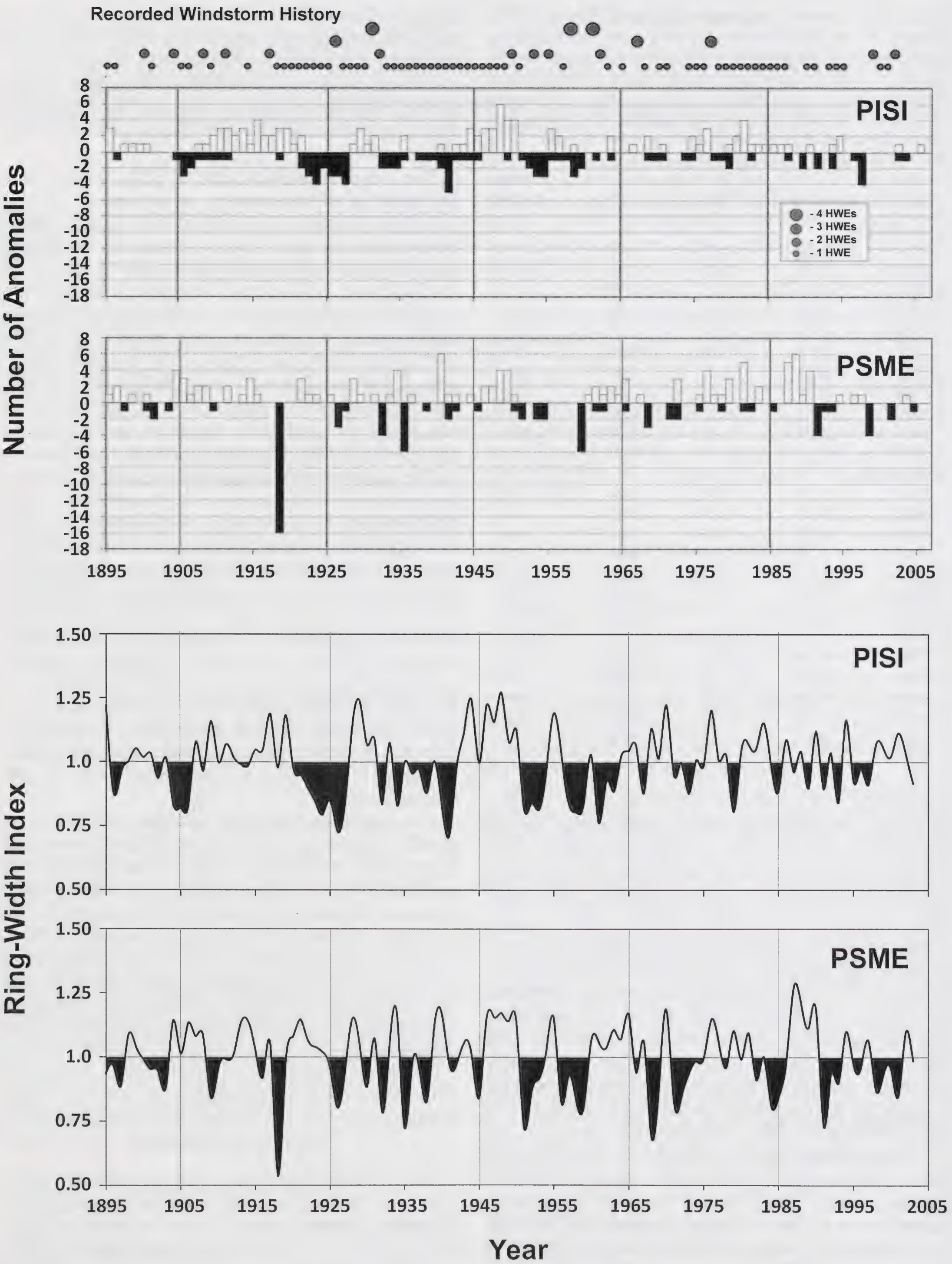
FIG. 4.   Population tree-growth anomalies and ring-width chronologies for Sitka spruce (PISI) and Douglas-fir (PSME) derived from standardized, unitless index values for the period of well-documented windstorms (1895–2003). (A) Percent of annual growth suppressions (>50% growth reduction; black bars) and releases (>50% growth increase; open bars); circles represent documented storms for the central Oregon coast during the historical period; larger circles indicate multiple storms (Range: 1–4 storms) in a given year (Source: Gibbs 1993; NOAA 2011; Knapp and Hadley 2012; Read 2013). (B) Ring-width chronologies illustrating population growth differences; shaded values represent < mean index values; non-shaded values > mean index values. Vertical lines added to facilitate visual comparisons.

Perpetua and documented windstorms during the historical era (1895–2003) is consistent with the number of HWEs previously reported for the entirety of the Oregon coast (Knapp and Hadley 2012). Conversely, we found Douglas-fir responded more reliably to storm clusters and high-magnitude events associated with MSYs (Fig. 4).

Both Sitka spruce and Douglas-fir are long-lived species (>600 years) capable of recording multiple growth episodes in response to canopy-changing events (Hadley and Knapp 2011). Although the long-term growth rates for Douglas-fir generally exceed those of Sitka spruce, our results suggest the greater shade tolerance, longer photo period, needle longevity, and canopy biomass of Sitka spruce (Minore 1979) promote a more rapid photosynthetic growth response following canopy disturbance and gap formation. Both Sitka spruce and Douglas-fir experience an early-age onset of radial growth suppressions and releases in response to local canopy gap conditions (Van Pelt and Franklin 1999; Deal et al. 2002).

The low suppression-to-release ratios exhibited by Sitka spruce and Douglas-fir suggests these species respond similarly to wind-induced canopy damage following HWEs. Growth suppression frequency—an indicator of canopy trauma and gap formation—thus appears to be a more useful metric for measuring the ecological significance of HWEs on local tree populations. This contention assumes suppression frequencies are largely dependent on the rate of canopy recruitment and tree populations sensitive and susceptible to canopy trauma. By contrast, release frequencies—the consequence canopy gap creation, gap size, gap orientation, and stand structure—are a common growth phenomenon among PNW tree species (e.g., Wright et al. 2000) discernible over the full demographic age range of trees from seedlings to remnant, canopy-dominant trees (Van Pelt and Franklin 1999; Deal et al. 2002; Hadley and Knapp 2011).

Evidence of large-scale, non-windstorm disturbance events are rare in our tree-ring record with the possible exception of the pronounced 1918 growth suppression we identify in our Douglas-fir chronology (Fig. 4). This growth event was contemporaneous with several regional climate and disturbance events including drought in southwestern Oregon (Graumlich 1987), an undocumented fire near the central coast (Morris 1934; Walstad et al. 1990; Impara 1997), and 1918 HWEs reported in south central Oregon (Weidman 1920) and northern Oregon (Gibbs 1993).

Our climate models indicate that the growth variations experienced by Sitka spruce and Douglas-fir at Cape Perpetua are unlikely to be related to regional annual temperature and precipitation. Pervious research also showed tree growth along the Oregon coast is statistically unrelated to ENSO conditions but weakly correlated to PDO conditions (Knapp and Hadley 2012). These results are similar to those reported by Nakawatase and Peterson (2006) who found tree growth in the Sitka spruce-western hemlock forests of Washington's Hoh River watershed unrelated to annual climatic variables. These findings are consistent with our hypothesis that coastal tree growth is strongly influenced by the near-annual occurrence of HWEs.

Windstorm Susceptibility

Several factors influence the susceptibility of Sitka spruce and Douglas-fir trees to wind disturbance at Cape Perpetua. Terrain is the most commonly cited factor contributing to the frequency and severity of wind disturbance across forest types (Everham and Brokaw 1996; Peterson 2000). Other factors influencing the windstorm susceptibility of Sitka spruce and Douglas-fir at Cape Perpetua include their proximity to the coastline, respective habitat requirements, relative sensitivities and tolerances to wind stress, and capacities for post-disturbance recovery.

The high incidence of growth anomalies relative to other coastal sites suggests Cape Perpetua's central coast location makes it highly vulnerable to the landfall of mid-latitude cyclones and typhoon remnants (Knapp and Hadley 2012). As a result, Cape Perpetua experiences more HWEs than southerly sites where Douglas-fir increases its dominance as a coastline species, more HWEs than northerly sites dominated by Sitka spruce, and a higher number of subregional storms traveling south to north and parallel to the coastline (Knapp and Hadley 2012).

The perpendicular alignment of the Cummins Creek and Gwynn Creek drainage basins relative to the coast (Fig. 1) appears to create wind corridors with little resistance to on-shore winds (cf. Everham and Brokaw 1996). The greater down-valley width of both drainages (Fig. 1) may also promote wind funneling (Kramer et al. 2001) exposing Sitka spruce to more frequent and higher velocity winds relative to those experienced by upslope and up-valley Douglas-fir. The ridges that define both drainage basins provide topographic protection from the southwesterly winds that have created massive blowdown elsewhere along the Oregon coast (Ruth and Yoder, 1953). These conditions (Peterson 2000; Kramer et al. 2001; Harcombe et al. 2004) may explain why trees in the Cummins Creek and Gwynn Creek drainages escaped the brunt of the Columbus Day Storm (Figs. 3 and 4) that leveled large adjacent areas of forest at other sites along the central Oregon coast (Lynott and Cramer 1966).

Topographic position also exerts a strong influence on habitat differentiation between Sitka spruce and Douglas-fir at Cape Perpetua and may be a secondary factor contributing to the frequency and severity of windstorm disturbance (Kramer et al. 2001). Occupying down-stream, low-elevation fluvial terraces and floodplains, Sitka spruce experiences a high incidence of wind-induced tree mortality and

high regeneration success facilitated by the presence of nurse logs (Harmon and Franklin 1989) and formation of canopy gaps (Harcombe 1986; Taylor 1990). Both Sitka spruce and Douglas-fir appear to benefit from canopy gap expansion following successive windstorms (e.g., Harcombe et al. 1994) but Sitka spruce requires the formation of larger canopy gaps (800–1000 m^2) relative to Douglas-fir ($\geq$750 m^2) (Spies and Franklin 1990) every several decades to persist at the stand scale (Taylor 1990).

Gap expansion appears to play an important role in the development of the horizontally-diverse (Franklin et al. 2002) "disturbance climax" (*sensu* Levin and Paine 1974) Sitka spruce community occupying the lower reaches of Cummins Creek (Franklin and Dyrness 1988; Wimberly and Spies 2001). By contrast, Douglas-fir occupies upslope, more fire-prone, inland landscape positions above Cummins and Gwynn Creek (Wimberly and Spies 2001) where it grows in well-drained, more nutrient-rich and finer-textured (higher density) soils (Hermann and Lavender 1990).

Maximum tree height, age and longevity, and growth rates are among the many biological factors influencing the growth susceptibility of Sitka spruce and Douglas-fir in high-wind environments (e.g., Lertzman et al. 1996; Sinton et al. 2000; Ott and Juday 2002). Tree height is widely accepted as an important factor influencing wind stress, canopy trauma, and blowdown of canopy trees (Everham and Brokaw, 1996). Old-growth Sitka spruce and Douglas-fir heights frequently exceed 70 m (e.g., Waring and Franklin 1979) consistent with the maximum post-windsnap tree (>80 m) and bole heights (>50 m) we recorded at our Cape Perpetua study site.

Differences in decay rates, fungal-induced heart-rot, root architecture, and heartwood density (Minore 1979; Hennon 1995; Larson and Franklin 2010) may also affect HWE susceptibility. Both Sitka spruce and Douglas-fir experience fungal-induced root and heart rot (Minore 1979) that contribute to their susceptibility to bole breakage under wind stress and ultimately influence the forest structure and regeneration dynamics (Hennon 1995). The delayed and prolonged influence of these pathogens (decades-centuries) may influence forest structure and the general susceptibility of previously treefall-scarred and infected trees to subsequent wind breakage or topple (Hennon 1995; Larson and Franklin 2010). Douglas-fir is more windthrow and windsnap resistant than Sitka spruce by virtue of its deeper root system and high heartwood specific gravity (Minore 1979).

CONCLUSIONS

Our results show both Sitka spruce and Douglas-fir provide an accurate, robust estimate of the incidence of mid-latitude windstorms but exhibit clear differences in their post-windstorm growth patterns. Sitka spruce displays a higher sensitivity and more individualistic response to windstorms than does Douglas-fir but exhibits a lower coincidence of growth anomalies concomitant with high-magnitude windstorms. Both species respond more frequently to canopy openings (accelerated growth) than to traumatic injury (growth reduction) with Sitka spruce again more responsive than Douglas-fir. These results are consistent with those reported for similar, single-species stands located ca. 350 km apart along the Oregon coast (Hadley and Knapp 2011).

While limited in scope, our case study provides a novel, hypothesis-generating perspective of HWEs and their relationship to the biogeography, autecology, and habitat requirements of Sitka spruce and coast Douglas-fire. Specifically, we posit that the local and regional distributions and persistence of Sitka spruce is predicated upon a mid-latitude windstorm regime that ensures the presence of canopy gaps, adequate light conditions, and CWD needed to sustain its continuous regeneration. Considered from a broader biogeographic perspective, the propensity of Sitka spruce to incur and thrive in a wind-disturbed environment—combined with its sensitivity to divergent selection (Holiday et al. 2011) and genetic variability (Xu et al. 2000; Sun et al. 2012)—is consistent with the "persistence niche" concept suggested for fire-adapted (Kaufmann 1990) and sprouting, disturbance-persisting plants (Bond and Midgley 2001). This view suggests Sitka spruce may hold an adaptive and competitive advantage over coast Douglas-fir on high-wind prone sites and may contribute to the local segregation of Sitka spruce and Douglas-fir.

HWEs represent an ecologically significant variable excluded from PNW climate characterizations and its correlative influence on the distribution of Sitka spruce and coast Douglas-fir (e.g., Farr and Harris 1979; Xu et al. 2000). While the influence of windstorms on the regional distribution of Sitka spruce remains vague, we believe a regional water-balance analysis (e.g., Stephenson 1998; Littel et al. 2008; Lutz et al. 2010) would help clarify the biogeographical significance of HWEs by providing a more precise estimate of Sitka spruce's drought tolerance (Watts et al. 1976), improving our understanding of coastal fire regimes (e.g., Stephenson 1998), and clarifying the effects of water-balance on regional tree-growth and wood density (Kantavichai et al. 2010). This research would ideally include the spatial and temporal characterization of regional fog regimes (e.g., Isaac 1946; Azevedo and Morgan 1974), the influence of fog on PNW coastal tree growth (cf. Burgess and Dawson 2004; Ewing et al. 2009), and the regional distribution of Sitka spruce and Douglas-fir in response to HWEs and fire during an era of shifting storm tracks (Yin 2005; Knapp and Hadley 2012), drought-related fires (cf. Long et al. 2002), and depleted fog regimes (Bakun 1990; Johnstone and Dawson 2010).

LITERATURE CITED

ARABAS, K. B., K. S. HADLEY, AND E. R. LARSON. 2006. Fire history of a naturally fragmented landscape in central Oregon. Canadian Journal of Forest Research 36:1108–1120.

AZEVEDO J. AND D. L. MORGAN. 1974. Fog precipitation in coastal California forests. Ecology 55:1135–1141.

BAKUN, A. 1990. Global climate change and intensification of coastal ocean upwelling. Science 247:198–201.

BERG, E. E., J. D. HENRY, C. L. FASTIE, A. D. DE VOLDER, AND S. M. MATSUOKA. 2006. Spruce beetle outbreaks on the Kenai Peninsula, Alaska, and Kluane National Park and Reserve, Yukon Territory: relationship to summer temperatures and regional differences in disturbance regimes. Forest Ecology and Management 227:219–232.

BIGLER, C., D. G. GAVIN, C. GUNNING, AND T. T. VEBLEN. 2007. Drought induces lagged tree mortality in a subalpine forest in the Rocky Mountains. Oikos 116:1983–1994.

BLACK, B. A., D. C. SHAW, AND J. K. STONE. 2010. Impacts of Swiss needle cast on overstory Douglas-fir trees of the western Oregon Coast Range. Forest Ecology and Management 259:1673–1680.

BOND, W. J. AND J. J. MIDGLEY. 2001. Ecology of sprouting in woody plants: the persistence niche. Trends in Ecology and Evolution 16:45–51.

BRIFFA, K. AND P. D. JONES. 1990. Basic chronology statistics and assessment. Pp. 137–162 in E. R. Cook and L. A. Kairiukstis (eds.), Methods of dendrochronology: applications in the environmental sciences. Kluwer Academic Publications, Dordrecht, The Netherlands.

BURGESS, S. S. O. AND T. E. DAWSON. 2004. The contribution of fog to the water relations of Sequoia sempervirens (D. Don): foliar uptake and prevention of dehydration. Plant, Cell & Environment 27:1023–1034.

BUSBY, P. E., C. D. CANHAM, G. MOTZKIN, AND D. R. FOSTER. 2009. Forest response to chronic hurricane disturbance in coastal New England. Journal of Vegetation Science 20:487–497.

COOK, E. R. AND R. L. HOLMES. 1986. Program ARSTAN. Version 6.05P. Available online at http://www.ltrr.arizona.edu/pub/dpl.

COUTTS, M. P. AND J. GRACE (eds). 1995. Wind and Trees. Cambridge University Press, Cambridge.

DEAL, R. L. AND J. C. TAPPEINER. 2002. The effects of partial cutting on stand structure and growth of western hemlock-Sitka spruce stands in southeast Alaska. Forest Ecology and Management 159:173–186.

EARLE, C. J. 2013. The Gymnosperm Database. Available online at http://www.conifers.org/ (accessed 8 October 2015).

EVERHAM, E. M., III, AND N. V. L. BROKAW. 1996. Forest damage and recovery from catastrophic wind. Botanical Review 62:113–185.

EWING, H. A., K. C. WEATHERS, P. H. TEMPLER, T. E. DAWSON, M. K. FIRESTONE, A. M. ELLIOTT, AND V. K. S. BOUKILI. 2009. Fog water and ecosystem function: heterogeneity in a California redwood forest. Ecosystems 12:417–433.

FARR, W. A. AND A. S. HARRIS. 1979. Site index of Sitka spruce along the Pacific Coast related to latitude and temperatures. Forest Science 25:145–153.

FOSTER, D. R. AND W. A. REINERS. 1986. Size distribution and expansion of canopy gaps in a northern Appalachian spruce-fir forest. Vegetatio 68:109–114.

FRANKLIN, J. F. AND C. DYRNESS. 1988. Natural vegetation of Oregon and Washington. Oregon State University Press, Corvallis, OR.

———, AND C. B. HALPERN. 2000. Pacific Northwest forests. Pp. 123–159 in M. G. Barbour and W. D. Billings (eds). North American terrestrial vegetation. Cambridge University Press, Cambridge, MA.

———, K. CROMACK JR., W. DENISON, A. MCKEE, C. MASER, J. SEDELL, F. SWANSON, AND G. JUDAY. 1981. Ecological characteristics of old-growth Douglas-fir forests. USDA Forest Service, General Technical Report PNW-118, Pacific Northwest Research Station, Portland, OR.

———, T. A. SPIES, R. VAN PELT, A. B. CAREY, D. A. THORNBURGH, D. R. BERG, D. B. LINDENMAYER, M. E. HARMON, W. S. KEETON, D. C. SHAW, K. BIBLE, AND J. CHEN. 2002. Disturbances and structural development of natural forest ecosystems with silvicultural implications, using Douglas-fir forests as an example. Forest Ecology and Management 155:399–423.

GIBBS, J. A. 1993. Pacific graveyard: a narrative of shipwrecks where the Columbia River meets the Pacific Ocean. Fourth edition. Binford & Mort Publishing. Portland, OR.

GRAUMLICH, L. J. 1987. Precipitation variation in the Pacific Northwest (1675–1975) as reconstructed from tree rings. Annals of the Association of American Geographers 77:19–29.

GRAY A. N., T. A. SPIES, AND M. J. EASTER. 2009. Microclimatic and soil moisture responses to gap formation in coastal Douglas-fir forests. Canadian Journal of Forest Research 32:332–343.

———, ———, AND R. J. PABST. 2012. Canopy gaps affect long-term patterns of tree growth and mortality in mature and old-growth forests in the Pacific Northwest. Forest Ecology and Management 281:111–120.

GREEN, S. E., P. A. HARCOMBE, M. E. HARMON, AND G. SPYCHER. 1992. Patterns of growth, mortality and biomass change in a coastal Picea sitchensis-Tsuga heterophylla forest. Journal of Vegetation Science 3:697–706.

GRISSINO-MAYER, H. D. 2001. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. Tree-Ring Research 57:205–221.

HADLEY, K. S. AND P. A. KNAPP. 2011. Detection of high-wind events using tree-ring data. Canadian Journal of Forest Research 41:1121–1129.

HALPERN, C. B. AND J. A. LUTZ. 2013. Canopy closure exerts weak controls on understory dynamics: a 30-year study of overstory-understory interactions. Ecological Monographs 83:221–237.

HARCOMBE, P. A. 1986. Stand development in a 130-year old spruce-hemlock forest based on age structure and

50 years of mortality data. Forest Ecology and Management 14:41–58.

———, S. E. GREENE, M. G. KRAMER, S. A. ACKERS, T. A. SPIES, AND T. VALENTINE. 2004. The influence of fire and windthrow dynamics on a coastal spruce-hemlock forest in Oregon, USA, based on aerial photographs spanning 40 years. Forest Ecology and Management 194:71–82.

HARMON, M. E. AND J. F. FRANKLIN. 1989. Tree seedlings on logs in *Picea-Tsuga* forests of Oregon and Washington. Ecology 70:48–59.

———, J. F. FRANKLIN, F. J. SWANSON, P. SOLLINS, S. V. GREGORY, J. D. LATTIN, N. H. ANDERSON, S. P. CLINE, N. G. AUMEN, J. R. SEDELL, G. W. LIENKAEMPER, K. CROMACK AND K. W. CUMMINS. 1986. Ecology of coarse woody debris in temperate ecosystems. Advances in Ecological Research 15:133–302.

HARRIS, A. S. 1990. *Picea sitchensis*. *In* Burns, R. M. and B. H. Honkala (tech coords). Silvics of North America. Volume 2. Conifers. USDA Forest Service, Agriculture Handbook 654. Website http://www.na.fs.fed.us/spfo/pubs/silvics_manual/Volume_1/picea/sitchensis.htm (accessed 13 April 2016).

HENNON, P. E. 1995. Are heart rot fungi major factors of disturbance in gap-dynamic forests? Northwest Science 69:284–293.

HERMANN, R. K. AND D. P. LAVENDER. 1990. *Pseudotsuga menziesii* (Mirb.) Franco. Douglas-fir. *In* Burns, R. M. and B. H. Honkala (tech coords). Silvics of North America. Volume 2. Conifers. Agriculture Handbook 654. Website http://www.na.fs.fed.us/spfo/pubs/silvics_manual/Volume_1/pseudotsuga/menziesii.htm (accessed 13 April 2016).

HOLLIDAY, J. A., H. SUREN, AND S. N. AITKEN. 2012. Divergent selection and heterogeneous migration rates across the range of Sitka spruce (*Picea sitchensis*). Proceedings of Royal Society B. 279:1675–1683.

HOLMAN, M. L. AND D. L. PETERSON. 2006. Sensitivity of forest growth to climatic variability in the Olympic Mountains, Washington. Canadian Journal of Forest Research 36:92–104.

HOLMES, R. L. 1983. Computer-assisted quality control in tree-ring dating and measurement. Tree-Ring Bulletin 43:69–78.

IMPARA, P. C. 1997. Spatial and temporal patterns of fire in the forests of the central Oregon Coast Range. Ph. D. dissertation. Oregon State University, Corvallis, OR.

ISAAC, L. A. 1946. Fog drip and rain interception in coastal forests. USDA Forest Service, Experimental Station Research Note 34, Pacific Northwest Research Station, Portland, OR.

ISHII, H. AND N. MCDOWELL. 2002. Age-related development of crown structure in coastal Douglas-fir trees. Forest Ecology and Management 169:257–270.

JOHNSTONE, J. A. AND T. E. DAWSON. 2010. Climatic context and ecological implications of summer fog decline in the coast redwood region. Proceedings of the National Academy of Science 107:4533–4538.

KANE, V. R., R. F. GERSONDE, J. A. LUTZ, R. J. MCGAUGHEY, J. D. BAKKER, AND J. F. FRANKLIN. 2011. Patch dynamics and the development of structural and spatial heterogeneity in Pacific Northwest forests. Canadian Journal of Forest Research 41:2276–2291.

KANTAVICHAI, R. D., BRIGGS, AND E. TURNBLOM. 2010. Modeling effects of soil, climate, and silviculture on growth ring specific gravity of Douglas-fir on a drought-prone site in western Washington. Forest Ecology and Management 259:1085–1092.

KAUFFMAN, J. B. 1990. Ecological relationships of vegetation and fire in Pacific Northwest forests. Pp. 39–52 *in* Walstad, J. D., S. R. Radosevich, and D. V. D. V. Sandberg, (eds.). Natural and Prescribed Fire in Pacific Northwest Forests. Oregon State University Press. Corvallis, OR.

KENNEDY, P. G. AND T. QUINN. 2001. Understory plant establishment on old-growth stumps and the forest floor in western Washington. Forest Ecology and Management 154:193–200.

KNAPP P. A. AND K. S. HADLEY. 2012. A 300-year history of Pacific Northwest windstorms inferred from tree rings. Global and Planetary Change 92–93:257–266.

KRAMER, M. G., A. J. HANSEN, M. L. TAPER, AND E. J. KISSINGER. 2001. Abiotic controls on long-term windthrow disturbance and temperate rain forest dynamics in southeast Alaska. Ecology 82:2749–2769.

LAFON, C. W. AND J. H. SPEER. 2002. Using dendrochronology to identify major ice storm events in oak forests of southwestern Virginia. Climate Research 20:41–54.

LARSON, A. J. AND J. F. FRANKLIN. 2010. The tree mortality regime in temperate old-growth forests: the role of physical damage. Canadian Journal of Forest Research 40:2091–2103.

LERTZMAN, K. P., G. D. SUTHERLAND, A. INSELBERG, AND S. C. SAUNDERS. 1996. Canopy gaps and the landscape mosaic in a coastal temperate rain forest. Ecology 77:1254–1270.

LEVIN, S. A. AND R. T. PAINE. 1974. Disturbance, patch formation, and community structure. Proceedings of the National Academy of Sciences 71:2744–2747.

LITTEL, J. S., D. L. PETERSON, AND M. TJOEELKER. 2008. Douglas-fir growth in mountain ecosystems: water limits tree growth from stand to region. Ecological Monographs 78:349–368

LONG, C. J. AND C. WHITLOCK. 2002. Fire and vegetation history from the coastal rain forest of the western Oregon Coast Range. Quaternary Research 58:215–225.

LUTZ, J. A. AND C. B. HALPERN. 2006. Tree mortality during early forest development: a long-term study of rates, causes, and consequences. Ecological Monographs 76:257–275.

———, J. W. VAN WAGTENDONK, AND J. F. FRANKLIN. 2010. Climatic water deficit, tree species ranges, and climate change in Yosemite National Park. Journal of Biogeography 37:936–950.

LYNOTT, R. E. AND O. P. CRAMER. 1966. Detailed analysis of the 1962 Columbus Day windstorm in Oregon and Washington. Monthly Weather Review 94:105–117.

MASS, C. AND B. DOTSON. 2010. Major extratropical cyclones of the Northwest United States: historical review, climatology, and synoptic environment. Monthly Weather Review 138:2499–2528.

MCDONALD, S. L. 2013. Understory diversity and succession on coarse woody debris in a coastal, old-growth forest, Oregon. M.S. Thesis. Portland State University, Portland, OR.

MINORE, D. 1979. Comparative autecological characteristics of northwestern tree species: a literature review. USDA Forest Service, General Technical Report PNW-87, Pacific Northwest Research Station, Portland, OR.

MORRIS, W. G. 1934. Forest fires in western Oregon and western Washington. Oregon Historical Quarterly 35:313–339.

NAKAWATASE, J. M. AND D. L. PETERSON. 2006. Spatial variability in forest growth - climate relationships in the Olympic Mountains, Washington. Canadian Journal of Forest Research 36:77–91.

NCDC. 2010. Monthly climatic data for Oregon Division 1. Website http://www7.ncdc.noaa.gov/CDO/CDODivisionalSelect.jsp# (accessed 16 May 2011).

———. 2011. Local climatological data publication for Newport and Tidewater, Oregon. Website http://www7.ncdc.noaa.gov/IPS/lcd/lcd.html (accessed 4 May 2011).

NOAA. 2011. Climate Prediction Center. Website http://www.cpc.ncep.noaa.gov/products/predictions/90day/fxus05.html2011 (accessed 18 February 2011).

OLIVER, C. D. 1981. Forest development in North America following major disturbances. Forest Ecology and Management 3:153–168.

ORVIS, K. H. AND H. D. GRISSINO-MAYER. 2002. Standardizing the reporting of abrasive papers used to surface tree-ring samples. Tree-Ring Research 58:47–50.

OTT, R. A. AND G. P. JUDAY. 2002. Canopy gap characteristics and their implications for management in the temperate rainforests of southeast Alaska. Forest Ecology and Management 159:271–291.

PABST, R. J. AND T. A. SPIES. 1999. Structure and composition of unmanaged riparian forests in the coastal mountains of Oregon. Canadian Journal of Forest Research 29:1557–1573.

PATOUX, J., X. YUAN, AND C. LI. 2009. Satellite-based midlatitude cyclone statistics over the Southern Ocean: 1. Scatterometer-derived pressure fields and storm tracking. Journal of Geophysical Research 114:1–15.

PETERSON, C. 2000. Catastrophic wind damage to North American forests and the potential impact of climate change. Science of the Total Environment 262:287–311.

READ, W. 2013. The Storm King: some historical weather events in the Pacific Northwest. Website http://www.climate.washington.edu/stormking/ (accessed 28 December 2013).

REBERTUS, A. J., T. KITZBURGER, T. T. VEBLEN, AND L. M. ROOVERS. 1997. Blowdown history and landscape patterns in the Andes of Tierra del Fuego, Argentina. Ecology 78:678–692.

RUTH, R. H. AND A. S. HARRIS. 1979. Management of western hemlock-Sitka spruce forests for timber production. USDA Forest Service, General Technical Report PNW-88, Pacific Northwest Research Station, Portland, OR.

———, AND R. A. YODER. 1953. Reducing wind damage in the forests of the Oregon Coast Range. USDA Forest Service, PNW Research Paper No. 7, Pacific Northwest Research Station, Portland, OR.

SCHAETZL, R. J., S. F. BURNS, D. L. JOHNSON, AND T. W. SMALL. 1989. Tree uprooting: review of impacts on forest ecology. Vegetatio 79:165–176.

SCHELHASS, M-J., G-J. NABUURS, AND A. SCHUCK. 2003. Natural disturbances in the European forests in the 19th and 20th centuries. Global Change Biology 9:1620–1633.

SINTON, D. S., J. A. JONES, J. L. OHMANN, AND F. J. SWANSON. 2000. Windthrow disturbance, forest composition, and structure in the Bull Run Basin, Oregon. Ecology 81:2539–2556.

SPEER, J. H. 2010. Fundamentals of tree-ring research. University of Arizona Press, Tucson, AZ.

———, T. W. SWETNAM, B. E., WICKMAN, and A. Youngblood. 2001. Changes in pandora moth outbreak dynamics during the past 622 years. Ecology 82:679–697.

SPIES, T. A. AND J. F. FRANKLIN. 1989. Gap characteristics and vegetation response in coniferous forests of the Pacific Northwest. Ecology 70:543–545.

———, J. F. FRANKLIN, AND M. KLOPSCH. 1990. Canopy gaps in Douglas-fir forests of the Cascade Mountains. Canadian Journal of Forest Research 20:649–658.

STAHLE, D. W., E. R. COOK, M. K. CLEAVELAND, M. D. THERRELL, D. M. MEKO, H. D. GRISSINO-MAYER, E. WATSON, AND B. H. LUCKMAN. 2000. Tree-ring data document 16th century megadrought over North America. Eos 81:121–125.

STAN, A. B. AND L. D. DANIELS. 2010. Growth releases of three shade-tolerant species following canopy gap formation in old-growth forests. Journal of Vegetation Science 21:74–87.

STATE OF OREGON. 2015. Natural Hazard Mitigation Plan. Chapter 2: Risk Assessment. Website https://www.oregon.gov/LCD/HAZ/docs/2015ORNHMP/2015ORNHMPApproved/Approved_2015ORNHMP_7_RA1.pdf. (accessed 13 April 2016).

STEPHENSON, N. L. 1998. Actual evapotranspiration and deficit: biologically meaningful correlates of vegetation distribution across spatial scales. Journal of Biogeography 25:855–870.

SUN, Y. -H., R. SHI, X. -H. ZHANG, V. L. CHIANG, AND R. R. SEDEROFF. 2012. MicroRNAS in trees. Plant Molecular Biology 80:37–53.

TAYLOR, A. H. 1990. Disturbance and persistence on Sitka spruce (Picea sitchensis [Bong.] Carr.) in coastal forests of the Pacific Northwest, North America. Journal of Biogeography 17:47–58.

VAN PELT. R. 2000. Influence of canopy structure on the understory environment in tall, old-growth, conifer forests. Canadian Journal of Forest Research 30:1231–1245.

——— AND J. F. FRANKLIN. 1999. Response of understory trees to experimental gaps in old-growth Douglas-fir forests. Ecological Applications 9:504–512.

——— AND S. C. SILLETT. 2008. Crown development of costal Pseudotsuga menziesii, including a conceptual model for tall conifers. Ecological Monographs 78:283–311.

VEBLEN, T. T., K. S. HADLEY, M. S. REID, AND A. J. REBERTUS. 1991. Methods of detecting past spruce beetle outbreaks in Rocky Mountain subalpine forests. Canadian Journal of Forest Research 21:242–254.

WALSTAD J. D., S. R. RADOSEVICH, D. V. SANDBERG (eds). 1990. Natural and prescribed fire in Pacific Northwest forests. Oregon State University Press, Corvallis, OR.

WARING, R. H. AND J. F. FRANKLIN. 1979. Evergreen coniferous forests of the Pacific Northwest. Science 204:1380–1386.

WATTS, W. R., R. E. NEILSON, AND P. G. JARVIS. 1976. Photosynthesis in Sitka spruce (Picea sitchensis [Bong.] Carr.): VII. Measurements of stomatal conductance and $^{14}CO_2$ uptake in a forest canopy. Journal of Applied Ecology 13:623–638.

WEIDMAN, R. H. 1920. The windfall problem in the Klamath Region, Oregon. Journal of Forestry 18:837–843.

WHITE, P. S. 1979. Pattern, process, and natural disturbance in vegetation. Botanical Review 45:229–299.

WIGLEY, T. M. L., K. R. BRIFFA, AND P. D. JONES. 1984. On the average value of correlated time series, with applications in dendroclimatology and hydrometeorol-

ogy. Bulletin of the American Meteorological Society 23:201–213.

WILSON, J. 2004. Vulnerability to wind damage in managed landscapes of the coastal Pacific Northwest. Forest Ecology and Management 191:341–351.

WIMBERLY, M. C. AND T. A. SPIES. 2001. Influences of environment and disturbance on forest patterns in coastal Oregon watersheds. Ecology 82:1443–1459.

WINTER, L. E., L. B. BRUBAKER, J. F. FRANKLIN, E. A. MILLER, AND D. Q. DeWITT. 2002. Initiation of an old-growth Douglas-fir stand in the Pacific Northwest: a reconstruction from tree-ring records. Canadian Journal of Forest Research 32:1039–1056.

WRCC. 2011. Western Regional Climate Center. Local climate summaries. Website http://www. wrcc. dri. edu/summary/climsmor. html (accessed 6 May 2011).

XU, P., C. C. YING, AND Y. A. EL-KASSABY. 2000. Multivariate analyses of causal correlation between growth and climate. Silvae Genetica 49:257–263.

YAMAGUCHI, D. K. 1991. A simple method for cross-dating increment cores from living trees. Canadian Journal of Forest Research 21:414–416.

YIN, J. H. 2005. A consistent poleward shift of the storm tracks in simulations of 21st century climate. Geophysical Research Letters 32:L18701.

FACTORS AFFECTING THE DISEASE SEVERITY OF ALTERNARIA BLACKSPOT IN NATURAL *BRASSICA RAPA* POPULATIONS ON THE CALIFORNIA AND OREGON COASTS

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Abstract

Fungal disease has important effects in natural and agricultural plant populations; however, we are still uncovering factors that influence severity in these systems. Understanding these factors is especially important because it has been predicted that climate change will increase fungal disease widely as a result of changes in precipitation. Here, we investigate the role of water availability and other ecological variables in determining disease severity of a foliar fungal disease. For this study, we focused on natural populations of an important annual, herbaceous plant, *Brassica rapa* and its fungal disease, Alternaria blackspot. We explored three hypotheses: (1) The factors that drive disease severity differ early in the growing season compared to late in the growing season. (2) Disease severity patterns for this fungus are driven by water availability to a greater extent than other ecological variables. (3) Disease severity increases with plant density. To address these hypotheses, data were collected in a spatially structured manner at two time points during the summer of 2011 from four *B. rapa* plant populations along the California and Oregon coasts. We found no clear factors drove disease severity early in the growing season, while factors such as host density, herbivory, sun exposure, and host developmental stage influenced disease severity later in the season. We found that soil moisture did not have a clear relationship with disease severity, and that greater host density supported greater disease severity. Our findings suggest that there are many factors influencing fungal disease, and the effects of these factors vary over the course of the growing season. These results have important implications for monitoring and predicting the effects of climate change on plant disease.

Key Words: Alternaria blackspot, *Brassica rapa*, disease, natural populations, water availability.

Plant pathogens are detrimental to plants, substantially reducing crop yield in agricultural systems, as well as influencing species distributions, population dynamics, community structure and evolution in natural systems (Burdon et al. 2006). The disease severity of foliar fungal pathogens is thought to be driven largely by water availability. However, disease dynamics in natural populations are exceedingly complex and factors such as host density, host developmental stage, and other microclimatic variables such as sunlight have been cited (Agrios 2005). Resolving the factors that drive disease severity by conducting multifactorial studies is an ongoing and high priority endeavor for both agricultural and natural plant systems (Pautasso et al. 2012; Thompson et al. 2014).

Since the environment plays a pivotal role in plant disease, recent research has focused on how climate change, including increases in temperature and altered precipitation patterns (IPCC 2014), will affect plant disease dynamics. Generally, climate change is expected to increase infectious disease in many plant ecosystems (Ayres 1984; Paul and Ayres 1987; Coakley et al. 1999; Garrett et al. 2014). Warmer temperatures or increased rainfall, which is predicted in some regions, might increase rates of pathogen growth and the size of vector populations (Patz et al. 2005), and water or temperature stress, which is predicted in other regions, might weaken host immunity (Desprez-Loustau et al. 2006).

In this study, we utilized natural plant populations, growing along a water availability gradient down the West Coast of the United States, to determine the relative importance across the growing season of water availability versus other host and ecological factors (i.e., host density, host developmental stage, herbivory, and sunlight) in determining disease severity of a foliar fungal pathogen. We focused on an economically important plant species, *Brassica rapa* L. (field mustard, Brassicaceae) and its

common foliar fungal disease Alternaria blackspot, which is caused by *Alternaria brassicae* (Berk.) Sacc. (1880) (Conn et al. 1990). This plant-pathogen system was chosen because *B. rapa* is an important crop species cultivated as seven diverse varietals ranging from leaf vegetables to oilseed crops (e.g., bok choi, napa cabbage, oilseed, turnip) (Rakow 2004). *Brassica rapa* is thought to have been brought to the U.S. from Europe for crop cultivation, and has since also formed feral populations with considerable genetic and phenotypic variation including variation in phenology. These populations inhabit a wide variety of environmental conditions, exhibit local adaptation, and are extremely evolvable (Franks et al. 2007). The pathogen, *A. brassicae*, is a common pathogenic sac fungus, which mainly spreads through rain splash, and causes damping off, leaf spots, and defoliation, and infects most cruciferous crops worldwide, severely decreasing crop yield (Rotem 1994; Meena et al. 2010). Many ecological factors affect Alternaria blackspot disease. Host density has been shown to have a positive relationship with disease levels, as has host developmental stage, with older plants being more diseased. In addition, rainfall/moisture has been shown to have large effects, with the highest levels of disease reported in areas of high rainfall (Humpherson-Jones and Phelps 1989; Rotem 1994; Agrios 2005; Meena et al. 2010; Nowicki et al. 2012).

In this study, we used natural populations of *B. rapa* to investigate the influence of multiple factors on disease severity, including time in the growing season, water availability, and spatial factors. Based on field observations and the literature, we formulated three main hypotheses: (1) The factors that drive disease severity differ early in the growing season compared to late in the growing season. (2) Disease severity patterns for this fungus are driven by water availability to a greater extent than other ecological variables (e.g., herbivory and sunlight). This would be supported at a regional scale by greater disease severity in areas with more rain, and at a finer scale by greater disease severity in quadratic plots with greater soil moisture. (3) Disease severity increases with plant density, with larger, denser patches that are closer together supporting greater disease severity. To explore these hypotheses, we collected data on ecological factors and measured Alternaria blackspot disease severity in four populations of *B. rapa* at two time points during the growing season of 2011.

MATERIAL AND METHODS

Field Locations

Field locations were chosen for this study along the California and Oregon coasts to represent a range of environmental variables, including rainfall. Two locations in central California (Muir Beach and Bodega Bay), and two locations in Oregon (Newport

and Cape Perpetua) were chosen. All field locations were on the coast and ranged from relatively dry in central CA (~100 cm/yr), to wetter in central Oregon (~200 cm/yr) based on precipitation data from 1961 to 1990 from NOAA Cooperative stations and USDA-NRCS SNOTEL stations (Daly et al. 1994) (Table 1). All locations also had considerable coastal fog and well-drained soil (USDA web soil survey 22 Feb 2012).

Sampling

Sampling was conducted towards the beginning (May) and end (July) of the 2011 *B. rapa* growing season, in a spatially structured manner (as described below), at all field locations, referred to by location codes CA1 for Muir Beach, CA2 for Bodega Bay, OR1 for Newport, and OR2 for Cape Perpetua (Table 1). Each population was mapped using an engineering compass and meter tape. The maps were then digitized and size of host patches and populations were determined in ImageJ (Schneider et al. 2012). To facilitate the analysis of patch size data, which had a few extreme outliers, patches were categorized into four size bins. Population size (total number of individuals) was estimated by multiplying density (see below) by patch size.

To explore the variation in Alternaria blackspot disease severity, we collected disease severity data from at least 10 quadrats in each population (see Table 2 for sample sizes), selected at random, from within host patches. Disease severity was measured as the percent of a quadrat with host tissue displaying symptoms, and was assessed visually by two independent researchers and averaged. Alternaria blackspot symptoms were clearly identifiable in the field, characterized by brown necrotic spots surrounded by chlorosis (Fig. 1). To verify that these symptoms were due to infection with *A. brassicae*, plant tissue was collected from all field locations for identification by spore morphology by the Plant Pathology Center at Oregon State University. Total pathogen load for a population was estimated by multiplying disease severity by patch size and summing across all patches.

To verify that the visual disease assessment was reliable, we conducted a greenhouse study (n = 576) in which we inoculated *B. rapa* accessions with field-collected *A. brassicae* under controlled conditions and measured resulting disease severity (O'Hara et al. in review). For this study, fresh spores were generated from field collected single spores by growing on carrot dextrose agar plates for a week, followed by carrot agar plates for another week (work permitted under APHIS license #P526P-11-00130). Fresh spores were strained through gauze to remove hyphae, and adjusted to a concentration of 1 × 10⁶ spores/mL in distilled water and 0.05% Tween. Ten μL of a fresh spore solution were applied to 2-wk-old leaves that had been wounded with a pipette tip. Control plants were wounded and treated with 10

TABLE 1. Characterization of field locations including precipitation and soil type. Precipitation data averaged over 10 yr was acquired from NOAA Cooperative stations and USDA-NRCS SNOTEL stations. Soil data was obtained from the USDA (USDA web soil survey Feb 22, 2012).

Location	Location info	ID	Region	Precipitation (cm/yr)	Soil type	Soil drainage
Muir Beach	37.863824 N, −122.573960 W	CA1	Central CA	101	Cronkhite (40%) and Barnabe (30%)	Moderately well-drained
Bodega Bay	38.313507 N, −123.061042 W	CA2	Central CA	101	Baywood loamy sand (85%)	Very well-drained
Newport	44.625044 N, −124.062710 W	OR1	Central OR	203	Neskowin-Salander silt loams (90%)	Well-drained
Cape Perpetua	44.282632 N, −124.109385 W	OR2	Central OR	203	Neskowin-Salander silt loams (90%)	Well-drained

FIG. 1. Characteristic Alternaria blackspot lesions on a *B. rapa* leaf. Photo by N. O'Hara.

µL of 0.05% Tween. Following inoculation, plants were kept at 90% humidity for 3 d and then placed at ambient humidity. The disease severities of the inoculated leaves for a subset of randomly selected plants (n = 277), including both control and infected, were scored 21 d post inoculation, using a visual index which ranged from 1 to 10 based on the amount of chlorosis and necrosis (Buchwald and Green 1992). Disease severity scores were independently verified by two researchers. Infected leaves displayed a highly significant increase in disease severity (one-way ANOVA comparing inoculated versus control plants: inoculated mean = 4.62 (±0.20), non-inoculated mean = 3.64 (±0.16), $F_{1,117}$ = 41.34, P < 0.0001), demonstrating that the spores that were isolated from the field were also causing the disease under greenhouse conditions.

We also quantitatively validated our visual index with a detached leaf assay (n = 50). Prior to inoculation, fully expanded leaves were detached from plants and placed in petri dishes on filter paper pre-moistened with distilled water and inoculated (as described above). Four days after inoculation, leaves were cleared, stained, and visualized through a microscope. Leaves were cleared by shaking overnight in a 1:3 acetic acid to ethanol solution, followed by a 1:5:1 acetic acid, ethanol, and glycerol solution. Leaves were rinsed in water, boiled in a solution of 5% Parker black ink and distilled white vinegar, and destained in vinegar acidified water, followed by a 5% vinegar wash (Vierheilig et al. 1998). Invading spores were counted at 100× magnification. Infected, stained leaves had an average of 9.5 (±8.7) spores per wound, while uninfected plants were free of symptoms and spores. We found that spore counts were correlated with the disease severity scores (Pearson correlation: r = 0.784, P = 0.0002).

To explore how ecological variables affect disease severity, we also collected the following field-data from the same patches for which disease severity data

TABLE 2. Characterization of populations by area. Area of population determined by mapping population, scanning and measuring in ImageJ. Host stems per m^2 and disease severity were calculated by averaging across quadrats at each location at the late time point. Standard deviations (SD) are given in parentheses.

Location	# of quadrats sampled	Total area of B. rapa (m^2)	# of host stems per m^2 ($\pm$SD)	Disease severity at late time ($\pm$SD)
CA1	18 early; 12 late	2,502.40	13.5 ($\pm$7.4)	35.2% ($\pm$32.7)
CA2	12 early; 11 late	1,415.90	20.6 ($\pm$15.3)	21.9% ($\pm$21.1)
OR1	13 early; 13 late	8,081.80	7.8 ($\pm$4.7)	26.5% ($\pm$21.1)
OR2	10 early; 10 late	139.4	4.3 ($\pm$2.1)	28.0% ($\pm$13.9)

was collected: host density (percent cover in each quadrat assessed visually), height of host (measured and averaged height in each quadrat), developmental stage of host (most common developmental stage in each quadrat classified as either young, flowering, fruiting, or senescing assessed visually), herbivory, (damage visually assessed), level of sun (visual assessment of amount of shade), and soil moisture in each quadrat (measured by TDR and verified gravimetrically as described below). Visual assessments were independently collected by two researchers and averaged. Distance between host patches was a spatial factor that was used in the analysis, and was determined by measuring the area of neighboring patches that fell within 3 m of the edge of each patch. We chose a 3 m distance because *Alternaria brassicicola*, a closely related fungus with similar means of dispersal, spreads by water splash primarily within that distance (Chen et al. 2003). Similar to patch size, distance between host patches had a few extreme outliers, so we grouped into five bins to facilitate our analysis: less than 12 m^2 of neighboring patches fell within 3 m of the patch of interest, 12–15 m^2, 15–18 m^2, 18–20 m^2, and >20 m^2.

To obtain measurements of soil moisture in each quadrat, we used a Field Scout TDR 100 Soil Moisture Meter (Spectrum Technologies, Inc.) with 10 cm probes. TDR readings were verified gravimetrically using a 25 cm soil core (Black 1965). There was a highly significant positive correlation between soil probe and gravimetric measurements so the soil probe, for which we had a more complete dataset, was used in all analyses (Pearson correlation: r = 0.63, P < 0.0001). All field data and soil moisture data were collected on the same day at each location. Because the time since the last rainfall varied between field locations, soil moisture measurements were not used to directly compare between populations, instead these data were analyzed using a nested design (see Analysis).

Analysis

To determine how disease severity was influenced by water availability and other factors over the growing season, two generalized linear mixed effects models (GLMMs) with location as a random effect were constructed, one for the early season collection, and one for the late. We chose to analyze our data using a mixed model because our experimental design was hierarchically nested by location, with multiple predictor variables collected from each location, so they were not entirely independent from each other. Separate early and late models were constructed because disease severity patterns and explanatory variables varied greatly between time points, and we were interested in exploring these patterns individually. Explanatory variables in the models included host density, soil moisture, host height, herbivory, and level of sun. Models were built to only include variables with strong literature support as being important factors in driving disease severity. For variables, such as host coverage and number of host stems per meter, which were highly correlated, we only included the variable that had the most biological support for being a possible explanatory variable; in this way did not include variables that were highly correlated. We used likelihood ratio tests to determine significance and degrees of freedom, by comparing models with and without each factor (Winter 2013). An FDR correction was conducted and uncorrected and corrected P values are reported. All analyses were conducted using R 3.0.1 (R Core Team 2013). Preceding analysis, data were transformed to meet model assumptions (Supp. Table 1). A Shapiro-Wilk Normality test was performed on all data following transformation to verify a normal distribution.

After fitting the full nested models, we were interested in further exploring the patterns of disease severity within each site at each time point. To determine how disease severity varied over time across locations (Hypotheses 1, 2), we conducted a 2-way ANOVA with location and time as fixed effects, and transformed disease severity data (Supp. Table 1) as the independent variable. A Tukey HSD post-hoc analysis was used to determine which locations were significantly different from each other. To determine if disease severity varied with soil moisture within locations (Hypothesis 2), we regressed disease severity on soil moisture for each location using transformed TDR values (Supp. Table 1), with early and late time points analyzed separately. An FDR correction was conducted and uncorrected and corrected P values are reported. To determine if disease severity varied with host density within locations (Hypothesis 3), we regressed disease severity on host density (coverage) for each location using transformed values (Supp. Table 1), with early and late time points analyzed separately, followed by an

FDR correction (both uncorrected and corrected P values are reported). We chose to focus on host coverage over patch size and distance between neighbors as a measure of host density in this analysis because coverage was at an appropriate scale for dispersion of the fungus (Humpherson-Jones and Phelps 1989; Rotem 1994; Nowicki et al. 2012).

RESULTS

Overall we found that Alternaria blackspot symptoms were widespread (Table 2), with 22.2 % of *B. rapa* individuals showing symptoms across the season. Disease severity varied widely and ranged from 21.9% of plants infected at Bodega Bay (CA2) to 35.2% infected at Muir Beach (CA1) at the late time point. Newport (OR1), which had by far the largest population of *B. rapa* (~8,082 m^2), also had by far the largest pathogen load with ~1,681 m^2 of host vegetation showing disease symptoms. We found that multiple ecological factors are statistically associated with increased disease severity of *A. brassicae* infection.

Different Factors Influence Disease Early and Late in the Growing Season

Addressing Hypothesis 1, we found that the factors influencing disease early versus late in the season differed. The early time point model explained 29.6% of the variation in disease severity, although no individual factors in the model were significantly correlated with disease severity. The late season model explained 58.3% of the disease severity variation observed. For this model we found that host density (% cover of host) significantly affected disease severity, with denser areas supporting greater disease severity (Beta = 0.009 ± 0.004; Table 3). We also found that herbivory and disease severity were negatively correlated and plants with greater herbivory damage displaying lower disease severity (Beta = –0.598 ± 0.161; Table 3). We found that size of host patch had a significant effect on disease severity with the largest patches having the greatest disease severity (Beta = 0.341 ± 0.225; Table 3). Level of sun had a significant effect on disease severity with quadrats with the lowest and highest levels of sun having the greatest disease severity (Beta lowest = 0.526 ± 0.206; Beta highest = 0.506 ± 0.109; Table 3). Distance between patches had a significant effect on disease severity with the very isolated patches having the greatest disease severity (Beta = 0.769 ± 0.281; Table 3). We also found that host developmental stage and disease severity were positively correlated with older plants displaying greater severity (Beta = 5.410 ± 1.991; Table 3).

We also found considerable variation in disease severity between the early and late time points. Disease severity was significantly higher at the later time point (2-way ANOVA and a post hoc Tukey's

TABLE 3. Results of GLMM models for early and late season exploring the factors affecting disease severity. Df and P values were calculated based on likelihood ratio tests (see text). Disease effect sizes (Beta estimates + SE) reported in text for significant variables. AIC early model = 52.7, late model = 21.7. Significant results are bolded.

	DF	Chisq	P value	FDR corrected P value
Early model				
Host coverage	1	0.388	0.534	0.712
Soil moisture (TDR)	1	0.012	0.914	0.983
Height	1	0.636	0.425	0.68
Herbivory	1	0.786	0.375	0.68
Patch size	3	4.831	0.185	0.68
Level sun	3	3.279	0.351	0.68
Distance between patches	3	7.034	0.071	0.568
Stage	1	0	0.983	0.983
Late Model				
Host coverage	**1**	**6.069**	**0.014**	**0.028**
Soil moisture (TDR)	1	0.353	0.553	0.553
Height	1	1.714	0.191	0.218
Herbivory	**1**	**11.671**	**0.0006**	**0.002**
Patch size	**3**	**7.502**	**0.024**	**0.032**
Level sun	**3**	**17.846**	**0.0001**	**0.0001**
Distance between patches	**3**	**7.808**	**0.02**	**0.032**
Stage	**1**	**6.247**	**0.012**	**0.028**
Error	19			

HSD test: $F_{1,91} = 9.8$, P = 0.003), with an increase in average disease severity from 17.1% tissue infected at the early time point to 28.0% at the later time point (Fig. 2A).

Soil Moisture, Host Coverage and Disease Severity

To address Hypothesis 2, that moisture availability affects disease severity, we examined these variables at a regional scale by comparing the sites, which had different amounts of rainfall, and at a local scale, by regressing disease severity on soil moisture within locations. While there was some variation among locations in rainfall, soil moisture, and disease severity (Tables 1 and 2), location and the interaction between time and location did not have a significant effect on disease severity (2-way ANOVA; Fig. 2B). At the local scale, the relationship between soil moisture and disease severity varied across field locations and time points and was not significant for any of these locations following an FDR correction. However, we saw a trend for the early Newport (OR1) time point and a significant relationship for the early Cape Perpetua (OR2) time point before the multiple test correction (Table 4). For Newport (OR1), soil moisture explained 24.4% of the variation and the relationship was negative, while for Cape Perpetua (OR2), soil moisture explained 54.7% of disease variation and the relationship was positive.

To address Hypothesis 3, that host cover affects disease severity, host cover was included as an explanatory variable in the GLMM model in
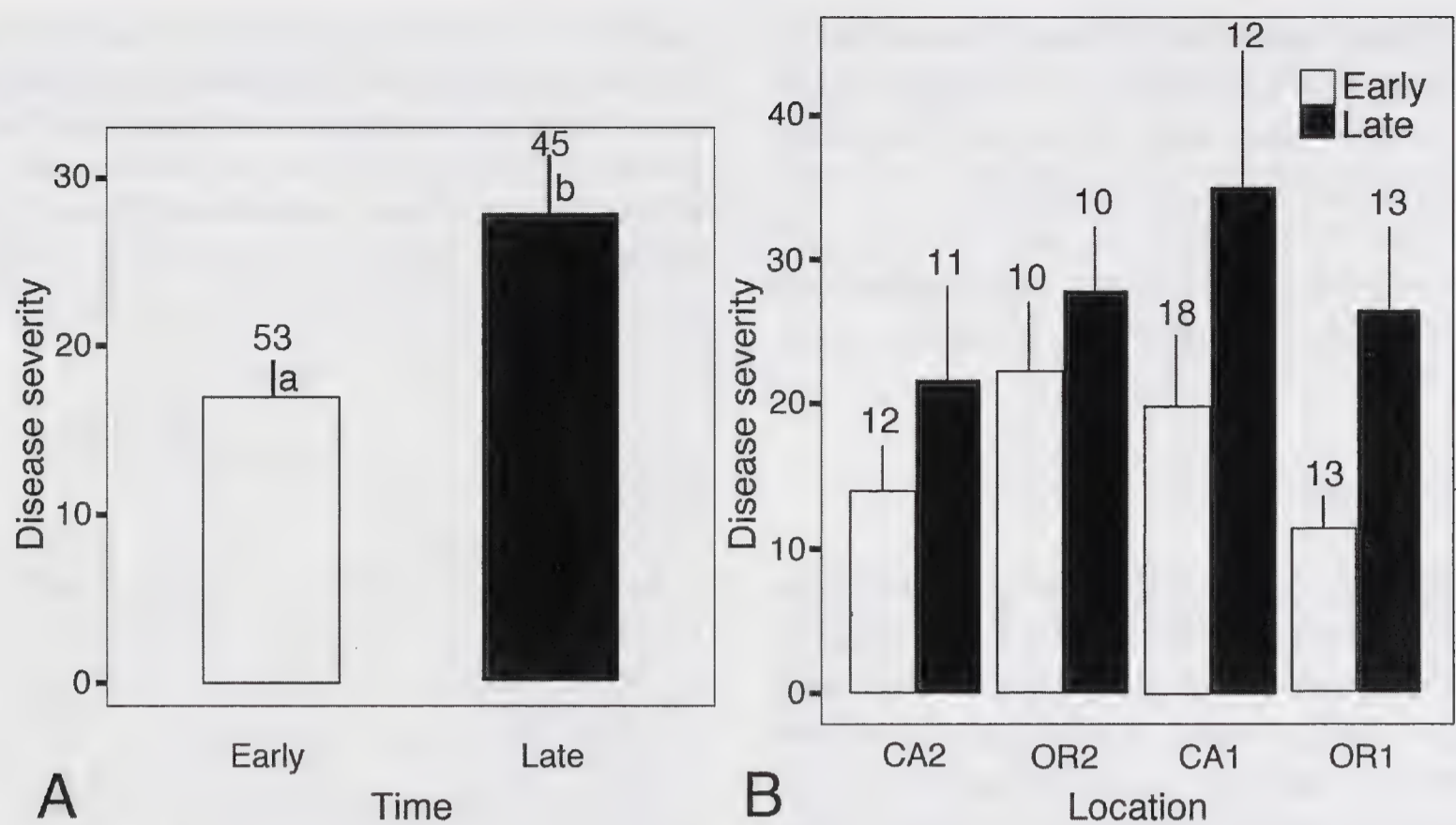
FIG. 2. Results of 2-way ANOVA with disease severity as independent variable and time and location as factors, followed by a post hoc Tukey's HSD test. Analysis conducted on log transformed disease severity data with untransformed data shown with (±SEM). A. Disease severity was significantly higher at the later time point, as indicated by the letters over the bars. B. Location at both early and late time points had no significant effect on disease severity. Sample sizes are shown above each bar.

addition to being explored within each site by regressing disease severity on coverage at each time point separately. In our GLMM analysis, we found that host cover had a significant effect on disease severity, with more dense areas supporting greater severity (reported above). According to the results from our within-location regressions, this relationship varied across field locations and time points and was significant for the CA2 location late in the season before a multiple test correction, but was only a trend after the correction (Table 5). Coverage was not significant for other locations at either time point (Table 5).

DISCUSSION

In this study, we found that disease severity varied widely with time, and several environmental and host factors. The models we created from data on these factors could explain approximately one-third to two-thirds of the variation in disease severity at two time points, respectively. Although this study was observational and does not definitively establish causation, the results provide important information on how a number of ecological factors vary with the severity of plant fungal disease.

Disease Severity Over Time

In testing Hypothesis 1, the effect of time on disease severity, we found that disease severity was significantly worse later in the season (Fig. 2), and that host developmental stage was significantly correlated with disease severity at the later time point (Table 3), a pattern common in disease studies (Madden and Hughes 1995). Studies on *Alternaria* fungal infection in *Brassica* species show that older plant tissue exhibits higher disease severity (Mridha and Wheeler 1993) and studies have shown that increases in disease severity occur later in the season due to weakened defenses at later developmental stages of the host plant (Rotem 1994). An alternative explanation is that the increase in severity over time is due to the prolonged length of time that the fungus has to establish and spread. While we cannot distinguish between these two explanations, we did find support for the role of developmental stage.

TABLE 4. Disease severity regressed on soil moisture taken by TDR. Regression was run on transformed data (Supplemental Table 1). Significant results (according to uncorrected or corrected P value) are bolded.

Location	Time	Sample size	Beta (SE)	T value	P value	FDR corr. P value	Multiple R^2
CA1	Early	18	−0.02 (0.02)	−1.08	0.297	0.594	0.067
	Late	12	−0.02 (0.02)	−1.26	0.237	0.594	0.137
CA2	Early	12	0.01 (0.01)	0.78	0.452	0.723	0.058
	Late	11	−0.01 (0.02)	−0.35	0.734	0.839	0.013
OR1	Early	13	−0.02 (0.01)	−1.89	0.086	0.344	0.244
	Late	13	0.002 (0.01)	0.17	0.866	0.866	0.003
OR2	**Early**	**10**	**0.02 (0.01)**	**3.11**	**0.015**	**0.12**	**0.547**
	Late	10	0.01 (0.02)	0.47	0.652	0.839	0.027

TABLE 5.   Disease severity regressed on host coverage. Regression was run on transformed data (Supp. Table 1). Significant results (according to uncorrected or corrected P value) are bolded.

Location	Time	Sample size	Beta (SE)	T value	P value	FDR corr. P value	Multiple R^2
CA1	Early	18	−0.0003 (0.004)	−0.07	0.948	0.948	0.0003
	Late	12	−0.02 (0.01)	−1.83	0.097	0.194	0.251
CA2	Early	12	0.003 (0.01)	0.51	0.622	0.829	0.025
	Late	**11**	**0.02 (0.01)**	**3.36**	**0.008**	**0.064**	**0.556**
OR1	Early	13	0.01 (0.004)	1.9	0.084	0.194	0.248
	Late	13	0.002 (0.01)	0.26	0.797	0.911	0.006
OR2	Early	10	0.01 (0.01)	1.39	0.201	0.322	0.195
	Late	10	0.02 (0.01)	2.15	0.064	0.194	0.37

Our two largest field locations, Muir Beach (CA1) and Newport (OR1), had the greatest area of infected tissue, particularly late in the season (Table 2). This suggests that a larger host population might support a greater amount of infection, however we would need many more locations included in the study to assess this systematically. This could be important when considering that feral crop plants, such as *B. rapa*, can act as a disease reservoir for closely related crops, such as canola (Burdon and Thrall 2008).

Although we cannot generalize across years because our data represents a single year, we can infer differences between early versus late season for the year we sampled. We found considerable differences in our early versus late season models. For the early time point we did not find a significant effect of any variables on disease severity. It is possible that during establishment of the fungus the factors measured do not have a major influence, or that the variation in disease was so low that we did not have enough power to detect associations. Our late season model had a much better goodness of fit and was able to explain about twice the variation in disease severity as the early model (~60%). Using this model we found that host density, host developmental stage, herbivory and level of sun all had significant effects on disease.

Overall, we found support for Hypothesis 1, which states that disease varies across the season and different variables mediate disease early versus late in the growing season. Timing could be an important consideration in conducting plant pathogen studies in natural populations.

Soil Moisture and Disease Severity

In exploring Hypothesis 2, we expected to see a positive correlation between disease severity and soil moisture in agreement with many disease studies, due to the fact that moisture is needed for the dispersal and growth of these fungal spores. Alternaria species have been shown to need high relative humidity in order to germinate, as well as water splash to disperse (Humpherson-Jones and Phelps 1989; Rotem 1994; Nowicki et al. 2012). Interestingly, soil moisture had no effect on disease severity either early or late in the full models, however we did see a positive association

using linear regression early in the growing season for one Oregon location (OR2), before a multiple test correction. This suggests that the relationship between moisture and disease depends on factors that vary among locations, and that water availability might not be driving disease severity in this plant pathogen system in many locations. Additionally, at the regional scale, we did not find greater disease in the wetter Oregon locations than in the drier Californian locations (Fig 2), although we would need to study more locations to test this.

Overall, we did not find support for Hypothesis 2. Instead, we found that other ecological variables (discussed below) played a greater role in influencing disease severity than water availability.

Host Density and Disease Severity

In exploring Hypothesis 3, we found a significant positive correlation between disease severity and host density (% cover) (Tables 3 and 5), consistent with literature reports that increased host density contributes to pathogen transmission (Burdon and Chilvers 1982). The density of the host did not have a significant effect in the early GLMM model, which suggests that density plays a greater role once infection has been established and is spreading, as one would expect later in the season.

Distance between patches also had a significant effect on disease severity in the late model, with the greatest disease severity found in patches that were the most isolated (Table 3), which is contrary to our expectations. We do not have a good explanation for this pattern, but it is possible that other factors correlated to patch distance, such as sun exposure or herbivory, could play a role.

Overall, we found support for Hypothesis 3, that host density played a role in disease severity with greater density supporting greater severity.

Other Ecological Factors and Disease Severity

The level of sun exposure had a significant effect on disease severity at the later time point (Table 3), and severity was highest for plants that received extreme amounts of sunlight (either very shaded or very high levels of sun). It is possible that the increase

in disease severity under low sun exposure is due to the inverse relationship between moisture and sun exposure, with a high level of moisture at the microclimate level supporting spore germination and spread. It is also possible that the increase in disease severity under high levels of sun exposure is due to an excess of light, which can stress plants and weaken plant defenses (Agrios 2005), however this would need to be tested experimentally in this system.

We found that herbivory had a highly significant and negative correlation with disease severity at the later time point (Table 3). This is contrary to many studies that have found that disease severity and herbivory are positively correlated (Kennedy and Barbour 1992; Simms and Rausher 1993). However, it is important to note that the relationship between disease severity and herbivore damage is complex and is mediated by many factors, including defensive compounds produced by the plant and food preference of the herbivores (Taiz and Zeiger 2006). In agreement with our finding, another study in *B. rapa*, exploring the relationship between herbivory by the gall midge and Alternaria fungal infection found that plants that had the highest level of fungal infection were preyed upon the least, which might have been due to food choice (Nakamura et al. 1995).

Conclusions

Over the course of a growing season the factors that drove disease severity of this foliar fungal pathogen varied, with no clear variables driving disease earlier in the season and host density, host developmental stage, level of sun exposure, and herbivory playing a role later in the season, when disease severity increased. This study illustrates that factors influencing disease severity in this natural plant pathogen system are complex, and that the timing of data collection and the study of multiple variables can be of great importance in understanding disease dynamics. Such complexity will have to be accounted for when developing models to predict the effects of environmental changes, such as climate change.

Acknowledgments

Literature Cited

Agrios, G. N. 2005. Plant pathology. Academic Press, Burlington, MA.

Ayres, P. G. 1984. The interaction between environmental stress, injury, and bioitic disease physiology. Annual Review of Phytopathology 22:53–57.

Black, C. A. 1965. Methods of soil analysis: Part I physical and mineralogical properties. American Society of Agronomy, Madison, WI.

Buchwald, L. and H. Green. 1992. Phytotoxicity of destruxin B and its possible role in the pathogenesis of Alternaria brassicae. Plant Pathology 41:55–63.

Burdon, J. J. and G. A. Chilvers. 1982. Host density as a factor in plant disease ecology. Annual Review of Phytopathology 20:143–166.

———, T. H. Peter, and L. Ericson. 2006. The current and future dynamics of disease in plant communitites. Annual Review of Phytopathology 44:19–39.

——— and P. H. Thrall. 2008. Pathogen evolution across the agro-ecological interface: implications for disease management. Evolutionary Applications:57–65.

Chen, L. Y., T. V. Price, and Z. Park-Ng. 2003. Conidial dispersal by Alternaria brassicola on Chinese cabbage (Brassica pekinensis) in the field and under simulated conditions. Plant Pathology 52:536–545.

Coakley, S. M., H. Scherm, and S. Chakraborty. 1999. Climate change and plant disease management. Annual Review of Phytopathology 37:399–426.

Conn, K. L., J. P. Tewari, and R. P. Awasthi. 1990. A disease assessment key for Alternaria blackspot in rapeseed and mustard. Canadian Plant Disease Survey 70:19–22.

Daly, D., R. Nielsen, and D. L. Philips. 1994. A statistical-topographic model for mapping climatological precipitation over mountainous terrain. Journal of Applied Meteorology 33:140–158.

Desprez-Loustau, M. L., B. Marcais, L. Nageleisen, D. Piou, and A. Vannini. 2006. Interactive effects of drought and pathogens in forest trees. Annals of Forest Science 63:597–612.

Franks, S. J., S. Sheina, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proceedings of the National Academy of Sciences 104:1278–1282.

Garrett, K., S. Thomas-Sharma, G. Forbes, and J. Nopsa. 2014. Climate change and plant pathogen invasions in L. Ziska, and J. Dukes, eds. Invasive species and global climate change. CABI.

Humpherson-Jones, F. M. and K. Phelps. 1989. Climatic factors influencing spore production in Alternaria brassicae and Alternaria brassicola. Annals of Applied Biology 114:449–458.

IPCC. 2014. Climate Change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contributions of Working Group II to the Fifith Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY USA.

Kennedy, G. G. and J. D. Barbour. 1992. Resistance variation in natural and managed systems. Pp. 12–41 in R. Fritz, and E. Simms, eds. Plant resistance to herbivores and pathogens. University of Chicago Press, Chicago.

Madden, L. V. and G. Hughes. 1995. Plant disease Incidence: distributions, heterogeneity, and temporal

analysis. Annual Review of Phytopathology 33:529–564.

MEENA, P. D., R. P. AWASTHI, C. CHATTOPADHYAY, S. J. KOLTE, AND A. KUMAR. 2010. Alternaria blight: a chronic disease in rapeseed-mustard. Journal of Oilseed Brassica 1:1–11.

MRIDHA, M. A. U. AND B. E. J. WHEELER. 1993. In vitro effects of temperature and wet periods on infection of oilseed rape by *Alternaria brassicae*. Plant Pathology 42:671–675.

NAKAMURA, R. R., T. MITCHELL-OLDS, R. S. MANASSE, AND D. LELLO. 1995. Seed predation, pathogen infection and life-history traits in *Brassica rapa*. Oecologia 102:324–328.

NOWICKI, M., M. NOWAKOWSKA, A. NIEZGODA, AND E. KOZIK. 2012. Alternaria black spot of crucifers: symptoms, importance of disease, and perspectieves of resistance breeding. Vegetable Crops Research Bulletin 76:5–19.

O'HARA, N. B., J. S. REST, AND S. J. FRANKS. 2016. Increased susceptibility to fungal disease is a cost of climate change adaptation in *Brassica rapa*. Evolution 70:241–248. doi: 10.1111/evo.12833

PATZ, J. A., D. CAMPBELL-LENDRUM, T. HOLLOWAY, AND J. A. FOLEY. 2005. Impact of regional climate change on human health. Nature 438:310–317.

PAUL, N. D. AND P. G. AYRES. 1987. Water stress modifies intraspecific interference between rust (*Puccinia lagenophorae* Cooke)-infected and healthy groundsel (*Senecio vulgaris* L.). New Phytologist 106:555–566.

PAUTASSO, M., T. DORING, M. GARBELOTTO, P. LORENZO, AND M. JEGER. 2012. Impacts of climate change on plant disease—opinions and trends. European Journal of Plant Pathology.

R DEVELOPMENT CORE TEAM. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

RAKOW, G. 2004. Species origin and economic importance of Brassica. Biotechnology in Agriculture and Forestry 54.

ROTEM, J. 1994. The genus *Alternaria*: biology, epidemiology, and pathogenicity. APS Press, St. Paul.

SCHNEIDER, C., W. RASBAND, AND K. ELICEIRI. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675.

SIMMS, E. L. AND M. D. RAUSHER. 1993. Patterns of selection on phytophagous resistance in *Ipomoea purpurea*. Evolution 47:970–976.

TAIZ, L. AND E. ZEIGER. 2006. Plant physiology. Sinauer, Sunderland, MA.

THOMPSON, S., S. LEVIN, AND I. RODRIGUEZ-ITURBE. 2014. Rainfall and temperature changes have confounding impacts on *Phytophthora cinnamomi* occurrence risk in the southwestern USA under climate change scenarios. Global Change Biology 20:1299–1312.

VIERHEILIG, H., A. P. COUGHLAN, U. WYSS, AND Y. PICHE. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Applied and Environmental Microbiology 64:5004–5007.

VLOUTOGLOU, I. AND S. N. KALOGERAKIS. 2000. Effects of inoculum concentration, wetness duration and plant age on development of early blight (*Alternaria solani*) and on shedding of leaves in tomato plants. Plant Pathology 49:339–345.

WINTER, B. 2013. Linear models and linear mixed effects models in R with linguistic applications. arXiv:1308.5499.

VARIATION IN OLD-GROWTH COAST REDWOOD (*SEQUOIA SEMPERVIRENS*) REFERENCE SITES IN MENDOCINO COUNTY, CALIFORNIA

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ABSTRACT

Restoration and management of old-growth conditions in coast redwood (*Sequoia sempervirens* [D. Don] Endl.) forests are traditionally based on an idealized set of characteristics that occur in productive stands. We compared three old-growth sites to quantify variability among remaining reference stands of the central coast redwood range in Mendocino County, California. Two of the sites are protected from coastal influence, have rich alluvial soils, and relatively high visitor usage. The third site is in close proximity to the coast with variable soil conditions and little visitor access. We randomly sampled twenty, 20-meter circular diameter plots in each site to evaluate basal area, tree density, species richness, canopy cover, shrub cover, and herbaceous species cover. We conducted multivariate analyses including nonmetric multidimensional scaling (NMDS), perMANOVA, and indicator species analysis (ISA) to examine the structural clustering and compositional metrics among the sites. Results indicated a strong separation among old-growth reference sites in the NMDS ordination and significant differences in sites using perMANOVA. The inland sites had high tree density, basal area, herbaceous understory cover, and cover of *Oxalis oregana* Nutt., *Adenocaulon bicolor* Hook., and *Viola glabella* Nutt. The coastal site had a high abundance of *Trillium ovatum* Pursh (an old-growth associated species), high shrub cover in canopy gaps, diverse species assemblages, and relatively high abundance of woodland-adapted perennial species. ISA provided a distinct suite of understory species for each site. The unique characteristics and high variability among these sites may offer a new, and potentially more accurate, standard for restoration and management.

Key Words: Coast redwood, indicator species, old-growth, understory diversity.

Coast redwood (*Sequoia sempervirens* [D. Don] Endl.) forests have been altered by human practices to the extent that less than 5% of the primeval forest remains (Noss 1999). Much of what remains, including some of the most dramatic stands, are preserved in parks and reserves. These iconic stands have traditionally been considered ideal reference sites for restoration and management (O'Dell 1996, Giusti 2007), while the variation inherent in less productive stands is less often considered. Though coast redwood reach their greatest growth potential on stable alluvial flats (Stone and Vasey 1968), the range of *Sequoia sempervirens* is characterized by topographic variability and can include areas of unstable and unproductive soil conditions (Madej 2011). Often coast redwood forests do not develop the extraordinarily large tree diameters and high species dominance found in the most archetypal stands, regardless of the specific site conditions (Russell and Woolhouse 2012). Yet old-growth remnants are increasingly rare, particularly in the central range, and thus local old-growth stands are underutilized when considering reference sites and developing restoration targets (Russell and Michels 2010; Russell et al. 2014). Due to the unique structural and physiological characteristics of *S. sempervirens* (Sawyer et al. 2000; Busing and Fujimori 2005; Sillett and Van Pelt 2007; Lorimer

et al. 2009; Madej 2010), reference sites from other forest types (e.g., Douglas-fir, mixed conifer-hardwood stands) yield few insights and may confound restoration objectives.

The dominance of herbaceous understory species in second-growth and old-growth stands varies (Table 1) by forest type and region (Duffy and Meier 1992; Jules and Rathcke 1999; Scheller and Mladenoff 2002). Pacific trillium (*Trillium ovatum* Pursh), for example, grows preferentially in old-growth Douglas-fir forests (Jules and Rathcke 1999) but at a lesser extent near timber harvest edges in coast redwood forests (Russell et al. 2000). In addition, shade adapted understory species such as calypso orchid (*Calypso bulbosa* [L.] Oakes), redwood violet (*Viola sempervirens* Greene), Douglas iris (*Iris douglasiana* Herb.), sugar scoop (*Tiarella trifoliata* L.), and vanilla leaf (*Achlys triphylla* [Sm.] DC.) grow favorably in old-growth stands and increase in abundance with time since harvest in second-growth stands (Russell et al. 2014). The fidelity of these species to old-growth conditions supports the assertion that understory herbaceous species may be indicative of disturbance intensity in previously harvested stands (Russell and Michels 2010). Yet, the distribution and dominance of herbaceous understory species is not uniform within old-growth sites; thus, understanding the variation in remaining

TABLE 1.   Summary of cited studies that examine variability in second-growth herbaceous understory.

Citation	Region	Plot scale	Study finding
Duffy and Meier 1992	Appalachian Mtns	1m × 1m quadrat	No difference in herbaceous cover noted between old-growth and second-growth stands.
Jules and Rathcke 1999	Siskiyou Mtns	Various (avg: 150m²)	*Trillium ovatum* recruitment decreased at old-growth forest edges.
Russell and Jones 2001	Coast redwood region	20m diameter	Understory cover higher in old-growth stands.
Scheller and Mladenoff 2002	Great Lakes region	2m × 2m quadrat	Statistically higher cover of shrubs in old-growth stands; all other taxonomic groups lower cover in old-growth. Differences in specific plants species not significant between groups.
Loya and Jules 2007	Coast redwood region	22.6m diameter	Understory species lowest in old-growth stands; three understory indicator species found in the old-growth stage.
Russell 2009	Coast redwood region	10m × 10 m quadrat	*Oxalis oregana, Athyrium filix-femina,* and *Vaccinium parviflorum* higher in older forest.
Russell and Michels 2010	Coast redwood region	20m diameter	Three coast redwood associated understory species favored on the older second-growth stands.
Russell et al. 2014	Coast redwood region	20m diameter	Two understory species (*Trillium ovatum* and *Viola sempervirens*) statistically higher in old-growth.

old-growth stands is essential for selecting prototypical reference sites.

The restoration paradigm in second-growth redwood stands currently promotes active management techniques (e.g., variable density thinning) to increase tree diameter, tree spacing, and the dominance of *Sequoia sempervirens* (O'Hara et al. 2010; Berrill et al. 2013). Although recovering second-growth stands do trend over time toward larger tree sizes, lower tree densities, and greater *S. sempervirens* species dominance (Russell and Michels 2010; Russell et al. 2014), defining restoration targets on short-term time scales (Foster et al. 1996) using iconic reference sites can lead to unrealistic expectations (Hilderbrand et al. 2005). Previous work in this part of the coast redwood range also noted high variation among sites (Michels and Russell 2010; Lambert 2012; Russell and Woolhouse 2012), yet the degree of this variation in remaining remnant old-growth stands in Mendocino County, California has yet to be quantified.

As such, we compared three remaining *S. sempervirens* old-growth reference sites in the central coast redwood range to examine variation in stand structure and species composition. We predicted that sites would exhibit significant differences in regard to stand density, basal area, and the cover of overstory (i.e., canopy), midstory (i.e., shrub), and understory (i.e., herbaceous) layers. We also predicted that significant variation among sites would manifest in understory indicators and in the abundance of individual understory species. Quantifying variation

among the remaining old-growth reference stands in this region may reveal nuanced structural features or subtle stand characteristics for direct implementation or consideration in ongoing restoration efforts.

MATERIALS AND METHODS

Study Areas

Study sites are located in Mendocino County in Northern California in the central part of the range of *S. sempervirens,* which extends in a narrow coastal band from Curry County, Oregon to Monterey County, California (Little 1971). The region's climate is characterized by warm, mild summers and cool, wet winters (Sawyer et al. 2000) with frequent moisture inputs from coastal summer fog (Burgess and Dawson 2004). Topography within study sites is complex and highly variable, with slopes exceeding 30° in some areas and elevation ranging from 55 meters (m) to 361 m. Vegetation is representative of the central redwood range with canopy species including coast redwood (*S. sempervirens*), western hemlock (*Tsuga heterophylla* [Raf.] Sarg.), Douglas-fir (*Pseudotsuga menziesii* [Mirbel] Franco var. *menziesii*), and tanoak (*Notholithocarpus densiflorus* [Hook. & Arn.] Manos et al.) (Giusti 2007; Russell and Michels 2010). Common understory species characteristic of this region include sword fern (*Polystichum munitum* [Kaulf.] C. Presl), huckleberry (*Vaccinium ovatum* Pursh), California rhododendron (*Rhododendron macrophyllum* D. Don), redwood

FIG. 1. Russell Unit (RU), Hendy Woods (HW), and Montgomery Woods (MW) old-growth coast redwood reference sites within Mendocino County, California.

sorrel (*Oxalis oregana* Nutt.), and western trillium (*Trillium ovatum*) (Russell and Michels 2010).

We selected old-growth reference sites using geographic information systems, land management history data (Rutland 2002), and regional knowledge of remaining old-growth stands in Mendocino County. Site selection criteria included old-growth stands (see Spies and Franklin 1996 for old-growth definition) dominated by *S. sempervirens*. We therefore selected the following three sites: 1) the 49-hectare (ha) Russell Unit, the smallest and most coastal of the three sites studied with nearly 24 ha of old-growth and residual old-growth forest located in the Brewery Creek watershed; 2) Montgomery Woods State Natural Reserve, a 462-ha alluvial redwood preserve located inland from the Russell Unit on Montgomery Creek; and 3) Hendy Woods State Park, a 342-ha preserve of old-growth coast redwood located in the Navarro River watershed (Fig. 1). All three sites are managed by California State Parks, and represent the largest remaining

unharvested redwood stands in Mendocino County, with the Russell Unit being one of the few sizable old-growth stands remaining on the Mendocino coast (R. Pasquinelli, California State Parks, personal communication).

Comparison of physiographic variables among sites indicated similar conditions with regard to precipitation and air temperature (Rittman and Thorson 2006), but some variation in distance to the coast, slope incline, and soil complex (Table 2). Edaphically, Hendy Woods and Montgomery Woods are on well-drained, sandy to loamy alluvial soils in the Gschwend-Frenchman soil complex (USDA, NRCS 2012), while the Russell Unit site consists primarily of poorly-drained marine terraces of the Ferncreek sandy loam complex (Rittiman and Thorson 2006; USDA, NRCS 2012). Human use and access also varies among the sites with well-developed facilities and trail systems in place in Hendy Woods and Montgomery Woods, but no such facilities in the Russell Unit.

TABLE 2. Characteristics of three old-growth reference sites in Mendocino County, California. Seasonal ranges of precipitation, temperature, and soil data from Rittiman and Thorson (2006).

	Russell Unit	Hendy Woods	Montgomery Woods
Distance to coast	~1 km	~20 km	~34 km
Mean annual precipitation	100 cm–165 cm	100 cm–205 cm	100 cm–205 cm
Mean annual air temperature	11°C–12°C	6°C–17°C	6°C–17°C
Soil complex	Poor to moderately-well drained, loam-sandy, loam-clay	Well drained, loam-sandy loam	Well drained, loam-sandy loam
Elevation range	99 m–127 m	55 m–62 m	253 m–361 m
Slope range	2°–34°	0°–10°	0°–4°
Facilities	No facilities, trails, parking	Campsites, day use, visitor center, trails	Trails
Management	Little to none	Facilities, trail use, aesthetic	Trail use, aesthetic
Dominant canopy species	*Sequoia sempervirens, Tsuga heterophylla,* or *Pseudotsuga menziesii* var. *menziesii*	*Sequoia sempervirens*	*Sequoia sempervirens*

Field Methods

We conducted a pilot study using the relevé method (Cain 1938) to determine plot size and sampling intensity. We randomly located twenty, 20-m diameter (0.031 ha), circular sample plots within each of the three study sites using ArcMap (ESRI 2011) for a total of 60 plots sampled. We located sample plots a minimum distance of 20 m from adjacent plots, 10 m from special habitats such as riparian areas and rock outcroppings, and 200 m from main access roads to reduce edge effects (Russell and Jones 2001).

At the center of each 20-m diameter plot, we recorded physiographic characteristics including location, slope, and aspect using a handheld global positioning device and a 360° azimuth pocket compass. At plot center we also estimated canopy cover with a convex spherical crown densiometer using cover estimates taken in each cardinal direction (Michels and Russell 2010). Within each 20-m plot, we collected overstory, midstory, and understory species-level data (Table 3) following nomenclature from the Jepson Manual (Baldwin et al. 2012; Jepson Flora Project 2015). We identified and measured tree species using circumference tape to calculate the diameter at breast height (1.37 m) of each individual. We identified and recorded seedlings as the number of tree species less than 1.37 m tall. We estimated cover of midstory and understory layers using percent cover classes (Gauch 1982) consistent with previous research conducted in this forest type (Russell and Michels 2010). We estimated species cover in 1% increments up to 5%, 5% increments up to 25%, and 10% increments up to 100%. We also included a sub 1% cover class of 0.5%.

Statistical Methods

We used a multivariate approach to compare site-level characteristics among the three old-growth sites including nonmetric multidimensional scaling (NMDS) (Kruskal 1964; Mather 1976), perMANOVA (Anderson 2001), and Indicator Species Analysis (ISA) (McCune and Mefford 2011). The set of input variables we analyzed for NMDS and perMANOVA included total tree density, total basal area, percent canopy cover, percent shrub cover, percent herbaceous cover, total species richness, and percent cover of common old-growth associate species *Trillium ovatum* (Loya and Jules 2007) and *Oxalis oregana* (Russell 2009, Michels and Russell 2012). We also calculated descriptive statistics on these data to compare with multivariate results. We further evaluated a suite of understory species using percent cover for the ISA.

NMDS provides a characterization analysis to visually illustrate clustering in large, multivariate datasets but does not provide a significance metric. In order to augment our preliminary interpretations of NMDS results, we used perMANOVA to isolate differences among sites. We used a Bray-Curtis distance measure for both the NMDS and perMANOVA analyses due to its robustness for community data (McCune and Grace 2002) and a Monte Carlo randomization test to confirm the strength of the NMDS output. Following initial NMDS analyses, we used the recommended two-dimensions from the analysis output with a stability criterion of 0.00001. Subsequently, we calculated Pearson and Kendall Correlation values among the original data and the NMDS output data to determine which of the variables, if any, were associated with differences among plots and probabilities of co-occurrences.

We used ISA to determine if the presence of rare flowering herbaceous species functioned as site indicators. ISA intuitively compares the relative abundance and relative constancy of species within groups using a Monte Carlo randomization to test significance (Dufrêne and Legendre 1997). This method allowed us to determine if any species drove species assemblages unique to a particular old-growth site. Since ISA evaluates rare indicators of a habitat type, common species found on all sites are

TABLE 3. Canopy, woody sub-canopy, and herbaceous species encountered in the Russell Unit (RU), Hendy Woods (HW), and Montgomery Woods (MW). "X" denotes presence at each site.

	RU presence	HW presence	MW presence
Canopy Species Observed			
Abies grandis (D. Don) Lindl.	X	—	—
Notholithocarpus densiflorus (Hook. & Arn.) Manos et al.	X	X	X
Pseudotsuga menziesii (Mirbel) Franco var. menziesii	X	—	X
Sequoia sempervirens (D. Don) Endl.	X	X	X
Tsuga heterophylla (Raf.) Sarg.	X	—	—
Umbellularia californica (Hook. & Arn.) Nutt.	—	X	—
Canopy Species Encountered	5	3	3
Woody Sub-Canopy Species Observed			
Gaultheria shallon Pursh	X	—	—
Lonicera hispidula (Lindl.) Torr. & A. Gray	X	—	X
Rhododendron macrophyllum D. Don	X	—	—
Rosa gymnocarpa Nutt.	—	X	X
Rubus leucodermis Torr. & A. Gray	—	X	X
Toxicodendron diversilobum (Torr. & A. Gray)	—	X	X
Vaccinium ovatum Pursh	X	—	X
Vaccinium parvifolium Sm.	X	—	—
Woody Sub-Canopy Species Encountered	5	3	5
Non-Flowering Herbaceous Species Observed			
Athyrium filix-femina (L.) Roth var. cyclosorum Rupr.	X	X	X
Blechnum spicant (L.) Roth	X	—	—
Polystichum munitum (Kaulf.) C. Presl	X	X	X
Pteridium aquilinum (L.) Kuhn var. pubescens Underw.	X	X	X
Woodwardia fimbriata Sm.	—	—	X
Non-Flowering Herbaceous Species Encountered	4	3	4
Flowering Herbaceous Species Observed			
Achlys triphylla (Sm.) DC.	—	X	X
Adenocaulon bicolor Hook.	—	X	X
Aquilegia formosa Fisch. ex DC.	—	X	X
Asarum caudatum Lindl.	X	X	X
Calypso bulbosa (L.) Oakes	X	—	X
Cardamine californica (Nutt.) Greene	X	—	—
Clintonia andrewsiana Torr.	X	—	—
Galium triflorum Michx.	X	X	X
Lysimachia latifolia (Hook.)	X	X	X
Maianthemum racemosum (L.) Link	X	—	X
Maianthemum stellatum (L.) Link	X	X	X
Oxalis oregana Nutt.	X	X	X
Poaceae spp.	X	X	X
Prosartes hookeri Torr.	X	X	X
Stachys mexicana Benth	X	X	—
Tiarella trifoliata L.	X	X	X
Trillium chloropetalum (Torr.) Howell	X	X	X
Trillium ovatum Pursh	X	X	X
Viola glabella Nutt.	—	X	—
Viola sempervirens Greene	X	X	X
Whipplea modesta Torr.	X	—	X
Flowering Herbaceous Species Encountered	18	16	17
Total Species Encountered	32	25	29

excluded from the ISA. We found eleven understory flowering (i.e., non-Pteridophytes) herbaceous species common to all sites (Table 4), and as a result, did not include these species in the analysis.

RESULTS

The three old-growth coast redwood stands differed widely in structure and composition. Descriptive comparisons of input variables among sites suggested differences in structural characteristics and ground-layer metrics. Canopy cover, tree density, herb cover, and *O. oregana* cover were highest in Hendy Woods, while species richness, shrub cover, and *T. ovatum* cover had highest mean values in the Russell Unit, and total basal area peaked in Montgomery Woods (Table 5).

NMDS ordination illustrated a clear distinction among the old-growth sites. Monte Carlo randomization tests were significant for each axis (P = 0.0196) and minimal overlap existed among the Russell Unit when compared to Hendy Woods and

TABLE 4. Combined total percent cover of understory species observed in old-growth reference sites: Russell Unit (RU), Hendy Woods (HW), and Montgomery Woods (MW). Asterisk (*) denotes species used in indicator species analysis.

Flowering herbaceous understory species	RU percent total cover	HW percent total cover	MW percent total cover
Achlys triphylla*	0	40	12
Adenocaulon bicolor*	0	12.5	35.5
Aquilegia formosa*	0	1	12
Asarum caudatum	4	17	0.5
Calypso bulbosa*	4.9	0	0.5
Cardamine californica*	16	0	0
Clintonia andrewsiana*	43	0	0
Galium triflorum	8.2	7.5	27.5
Lysimachia latifolia	6.1	1.5	17.5
Maianthemum racemosum*	8	0	2
Maianthemum stellatum	1.5	8	6
Oxalis oregana	135	780	481
Poaceae spp.	15	28.5	58.5
Prosartes hookeri	7.5	61.5	23
Stachys mexicana*	1	34	0
Tiarella trifoliata	44.5	11.5	54.5
Trillium chloropetalum	6.5	5	18.5
Trillium ovatum	31.5	10.5	22
Viola glabella*	0	29	27
Viola sempervirens	35.5	4.5	21.5
Whipplea modesta*	6	0	5.5

Montgomery Woods (Fig. 2). As illustrated by the NMDS, most variation manifested among sites. Pearson Correlations (Table 6) (r > |0.500|) and the NMDS ordination characterized the Russell Unit as having high values of shrub cover, canopy cover, species richness and low values of O. oregana cover and total herb cover. Weaker correlations (r > |0.300|) indicated tree density and T. ovatum cover also reflected Russell Unit characteristics. Results of the perMANOVA ($F_{1,60}$ = 22.176, P = 0.002) supported the NMDS analysis and Pearson Correla-

tions findings. Pairwise comparisons among Montgomery Woods and Hendy Woods (P = 0.0046), Montgomery Woods and the Russell Unit (P = 0.0002), and the Russell Unit and Hendy Woods (P = 0.0002), indicated significant differences in variance among sites.

ISA for understory species demonstrated four indicator species for the Russell Unit, four indicator species for Hendy Woods, and six indicator species for Montgomery Woods (Table 7). Significant differences existed among groups of species that reached maximum indicator value within each site. Clintonia (Clintonia andrewsiana Torr.) had the highest ISA value for the Russell Unit, hedgenettle (Stachys mexicana Benth.) had the highest ISA value in Hendy Woods, and trail plant (Adenocaulon bicolor Hook.) had the highest ISA value for Montgomery Woods.

Qualitative observations (Table 2) support the findings that variation manifested between old-growth sites, especially when comparing the Russell Unit to Montgomery Woods and Hendy Woods. Yet, although the Russell Unit sample plots were generally more similar to one another than plots sampled in other sites, variation also existed in each site. Within the Russell Unit, the soil complex and dominant canopy species encountered was more variable. In this coastal site, soils varied from poorly drained to moderately-well drained and loam-sandy to loam-clay. In contrast, Montgomery Woods and Hendy Woods largely consisted of well-drained, loam to sandy loam soils. Further, qualitative observations of dominant canopy species varied within the Russell Unit, predominated by Sequoia sempervirens, Tsuga heterophylla, or Pseudotsuga menziesii var. menziesii. At Montgomery Woods and Hendy Woods, the dominant canopy species observed was Sequoia sempervirens throughout the sample plots. Topography within the Russell Unit was also more variable, ranging between 2° and 34° slopes, when compared to Hendy Woods (slopes did

TABLE 5. Descriptive characteristics (mean and standard error) of the three old-growth reference sites: Russell Unit (RU), Hendy Woods (HW), and Montgomery Woods (MW).

|	RU mean (±SE)	HW mean (±SE)	MW mean (±SE)
Canopy Metrics			
Tree density (trees/plot)	13.8 (1.3)	36.0 (5.6)	18.8 (2.0)
Basal area (m2/ha)	5.1 (0.7)	12.9 (1.5)	15.7 (1.9)
Canopy cover (percent/plot)	84.6 (0.9)	88.3 (0.6)	84.1 (0.9)
Understory Metrics			
Shrub cover (percent/plot)	45.5 (5.0)	7.5 (0.9)	19.2 (4.0)
Herb cover (percent/plot)	15.6 (2.2)	46.8 (4.0)	40.3 (4.3)
Trillium ovatum cover (percent/plot)	1.6 (0.2)	0.5 (0.1)	1.1 (0.2)
Oxalis oregana cover (percent/plot)	6.8 (1.4)	39.0 (4.6)	24.1 (3.9)
Seedling density (seedlings/plot)	6.9 (1.2)	12.8 (2.2)	64.9 (12.9)
Sequoia sempervirens seedling density (seedlings/plot)	0.3 (0.2)	4.8 (1.8)	54.7 (12.0)
Diversity Metrics			
Total richness (species/plot)	17.8 (0.8)	12.9 (0.7)	15.6 (0.8)
Shrub richness (shrub species/plot)	6.0 (0.2)	3.0 (0.3)	4.6 (0.3)
Herbaceous richness (herb species/plot)	8.4 (0.8)	7.6 (0.5)	9.6 (0.7)
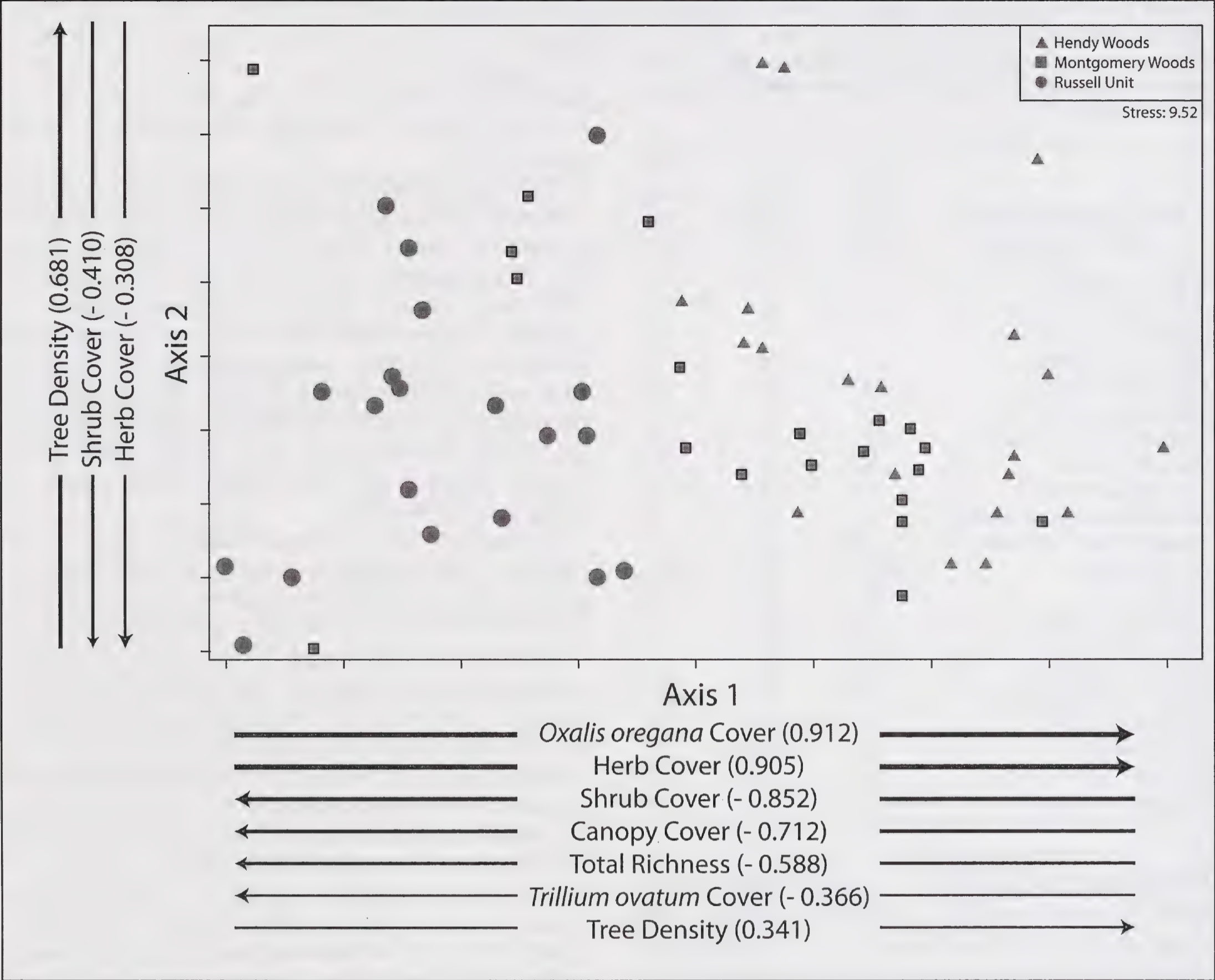
FIG. 2.   Nonmetric multidimensional scaling main output for old-growth variables. Direction of influence (arrow direction) and strength (arrow weight) for variables with r > |0.300| indicated on each axis.

not exceed 10°) and Montgomery Woods (slopes did not exceed 4°).

DISCUSSION

The variation among these stands indicates greater landscape diversity and structural heterogeneity than is generally recognized in coast redwood old-growth forests. A variety of mechanisms may have led to the observed distinctions among these regional sites (i.e., microclimates, disturbance regimes, edaphic conditions, public access). Nonetheless, these findings still offer useful insights for current restoration paradigms.

Some of the variation found among these sites is likely explained by the Russell Unit's close proximity to the Pacific Ocean, which results in more frequent natural disturbances associated with coastal locations such as blowouts, salt spray, and strong winds (Wu and Guo 2006; Lorimer et al. 2009). These reoccurring, stochastic disturbances likely led to the openings of small canopy gaps and associated recruitment of otherwise suppressed species, as

illustrated by the Russell Unit's high overall species richness yet low values in total understory cover, tree density, and basal area. Such gap disturbances increase niche partitioning and nutrient availability, allowing for a diverse assemblage of species to

TABLE 6.   Pearson and Kendall correlations with ordination axes for stand structure and floristic data collected on the three coast redwood old-growth references sites in Mendocino County, California.

Variable	Axis 1 Pearson correlation (r)	Axis 2 Pearson correlation (r)
Percent *Oxalis oregana* cover	0.912	−0.289
Percent herb cover	0.905	−0.388
Percent shrub cover	−0.852	−0.41
Percent canopy cover	−0.712	0.443
Total species richness	−0.588	0.166
Total tree density	0.341	0.681
Basal area (m²)	0.25	0.237
Percent *Trillium ovatum* cover	−0.366	−0.058

TABLE 7. Indicator species analysis comparisons on understory herbaceous species in the three old-growth reference sites in Mendocino County, California. Asterisk (*) denotes species with the highest indicator value in each stand.

	Indicator value	P-value
Russell Unit Indicator Species		
Calypso bulbosa	59	0.0002
Cardamine californica	55	0.0002
*Clintonia andrewsiana**	85	0.0002
Maianthemum racemosum	20	0.0534
Hendy Woods Indicator Species		
Achlys triphylla	42.3	0.0018
*Stachys mexicana**	68	0.0002
Viola glabella	41.4	0.0012
Montgomery Woods Indicator Species		
Galium triflorum	57.3	0.0002
*Adenocaulon bicolor**	59.2	0.0002
Aquilegia formosa	46.2	0.0002

TABLE 8. Potential qualitative characteristics of coastal old-growth sites and inland old-growth sites including representative photographs; symbols indicate high (+) or low (−) relative values. "0" indicates species not found in site(s).

Russell Unit	Site characteristic	Montgomery Woods and Hendy Woods
+	Species richness	−
+	Shrub cover	−
+	*Trillium ovatum* cover	−
+	*Clintonia andrewsiana* cover	0
−	Basal area	+
−	Tree density	+
−	Herbaceous cover	+
−	*Oxalis oregana* cover	+
−	*Stachys mexicana* cover	+
0	*Adenocaulon bicolor* cover	+
0	*Viola glabella* cover	+

flourish. These patterns of patch development are commonly observed in other old-growth stands in the Pacific Northwest (Wimberly 2002; van Mantgem and Stuart 2012).

While shade tolerant, old-growth associated species, such as *O. oregana* and *T. ovatum*, are present in the understory of each old-growth stand, *O. oregana* tended to dominate in Montgomery Woods and Hendy Woods (Sawyer et al. 2000; Loya and Jules 2007; Russell and Michels 2010). *Oxalis oregana*, a common species in coniferous forests (Russell and Michels 2010), is a strong competitor using vegetative spread via underground rhizomes as its main method of reproduction (Baldwin et al. 2012). In fact, most of the understory indicator species on each site (Table 7) are perennial, rhizomatous species found in moist, shady forests (Baldwin et al. 2012). The fertile alluvial soils of Hendy Woods and Montgomery Woods (Rittiman and Thorson 2006) in combination with low frequency of shrub canopy formation, is seemingly ideal for the proliferation of shade tolerant, moisture adapted species such as *O. oregana*.

While Montgomery Woods and Hendy Woods are both characterized by rich alluvial soils, nutrient-poor uplifted marine terraces also persist on the Russell Unit (Russell and Woolhouse 2012), which may account for some of the understory species variation among sites. Heavy shrub cover, common on marine terraces, reached maximum values in the Russell Unit. In addition, some species with significant ISA values observed in the Russell Unit, such as toothwort (*Cardamine californica* [Nutt.] Greene) and false Solomon's seal (*Maianthemum racemosum* [L.] Link) are most common in canopy gaps and open woodlands (Baldwin et al. 2012). The presence of species that prefer open woodlands may be related to edaphic conditions on the Russell Unit site.

Although the Russell Unit can be described as a site with a high degree of natural disturbance, the

cover of *T. ovatum*, a species known to be sensitive to human disturbance (Loya and Jules 2007), was in higher abundance than on the other two sites. This finding suggests that natural and anthropogenic disturbances are not analogous. Both Hendy Woods and Montgomery Woods are managed for a high volume of visitor use, while the Russell Unit is not readily accessible to the public (i.e., no facilities, parking, or managed trails).

These findings support our hypothesis that significant variation exists among remaining old-growth reference sites in the central coast redwood region. Thus, we developed a qualitative matrix of potential indicators for potential application in restoration efforts (Table 8). Archetypal stands similar to Montgomery Woods and Hendy Woods may have relatively high tree density and basal area; herbaceous understory cover; cover of vegetatively spreading, shade tolerant understory species such as *Oxalis oregana*; and cover of perennial herbs including *Adenocaulon bicolor*, *Viola glabella*, and *Stachys mexicana*. In heterogeneous stands akin to the Russell Unit, dominant characteristics may include high cover values of sensitive understory species (*Trillium ovatum*), perennial *Clintonia andrewsiana* and other woodland-adapted perennial species, high shrub cover in canopy gaps, and diverse species assemblages. Potentially, incorporating these understory characteristics and other more subtle features of remaining old-growth stands will provide insight for restoration managers recognizing high variation in reference sites.

False assumptions of uniformity confound restoration targets. Thus, local site characteristics should be taken into consideration when designing active restoration projects. In developing future restoration efforts, biodiversity impacts to sensitive ground-layer species, overall species richness, and stand complexity should be carefully studied. Considering local stand variation could provide land use managers with additional references sites for comparison, more

realistic restoration expectations, and a dynamic template for coast redwood restoration.

We recognize these findings represent a case-study scenario, and require augmentation for large-scale application. Although this study identifies differences in the few remaining old-growth coast redwood stands localized in Mendocino County, other stands may exist in this region or elsewhere that would further illustrate variation within this forest type. Additional research on other coast redwood old-growth stands would provide a stronger foundation to extrapolate these results. It is also important to note that causality among physical characteristics and biotic variables was beyond the scope of this study. Yet, the application of multivariate analyses, coupled with evidence from this case study in Mendocino County, reveals new insights for management of old-growth and recovering second-growth sites.

LITERATURE CITED

ANDERSON, M. J. 2001. A new method of non-parametric multivariate analysis of variance. Austral Ecology 26:32–46.

BALDWIN, B. G., D. H. GOLDMAN, D. J. KEIL, R. PATTERSON, T. J. ROSATTI, AND D. H. WILKEN (eds.). 2012. The Jepson Manual: vascular plants of California, Second Edition. University of California Press, Berkeley, CA.

BERRILL, J.-P., C. B. BEAL, D. H. LAFEVER, AND C. M. DAGLEY. 2013. Modeling young stand development towards the old-growth reference condition in evergreen mixed-conifer stands at Headwaters Forest Reserve, California. Forests 4:455–470.

BURGESS, S. S. O AND T. E. DAWSON. 2004. The contribution of fog to the water relations of *Sequoia sempervirens* (D. Don): foliar uptake and prevention of dehydration. Plant Cell & Environment 27:1023–1034.

BUSING, R. T. AND T. FUJIMORI. 2005. Biomass, production and woody detritus in an old coast redwood (*Sequoia sempervirens*) forest. Plant Ecology 177:177–188.

CAIN, S. A. 1938. The species-area curve. American Midland Naturalist 19:573–581.

DUFFY, D. C. AND A. J. MEIER. 1992. Do Appalachian herbaceous understories ever recover from clearcutting? Conservation Biology 6:196–201.

DUFRÊNE, M. AND P. LEGENDRE. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 61:53–73.

ESRI. 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.

FOSTER, D. R., D. A. ORWIG, AND J. S. MCLACHLAN. 1996. Ecological and conservation insights from reconstructive studies of temperate old-growth forests. Trends in Ecology & Evolution 11:419–424.

GAUCH, H. G., JR. 1982. Multivariate analysis in community ecology. Cambridge University Press, Cambridge, UK.

GIUSTI, G. 2007. Structural characteristics of an old-growth coast redwood stand in Mendocino County, California. Pp. 161–168 *in* R.B. Standiford, G.A. Giusti, Y. Valachovic, W.J. Zielinski, and M.J. Furniss (eds.), Proceedings of the redwood region forest science symposium: what does the future hold? U.S. Department of Agriculture, U.S. Forest Service. PSW-GTR-194, Albany, CA.

HILDERBRAND, R. H., A. C. WATTS, AND A. M. RANDLE. 2005. The myths of restoration ecology. Ecology and Society 10: 19.

JEPSON FLORA PROJECT (EDS.). 2015. *Jepson eFlora*. Website http://ucjeps.berkeley.edu/IJM.html (accessed 2 December 2015).

JULES, E. S. AND B. J. RATHCKE. 1999. Mechanisms of reduced trillium recruitment along edges of old-growth forest fragments. Conservation Biology 13:784–793.

KRUSKAL, J. B. 1964. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29:1–27.

LAMBERT, Z. R. 2012. A descriptive study of old-growth *Sequoia sempervirens* growth form and canopy structure on a pygmy forest ecotone in Mendocino County. M.S. Thesis. San José State University, San Jose, CA.

LITTLE, E. L., JR. 1971. Atlas of United States trees, volume 1, conifers and important hardwoods: U.S. Department of Agriculture Miscellaneous Publication 1146.

LORIMER, C. G., D. J. PORTER, M. A. MADEJ, J. D. STUART, S. D. VEIRS JR., S. P. NORMAN, K. L. O'HARA, AND W. L. LIBBY. 2009. Presettlement and modern disturbance regimes in coast redwood forests: implications for the conservation of old-growth stands. Forest Ecology and Management 258:1038–1054.

LOYA, D. T. AND E. S. JULES. 2007. Use of species richness estimators improves evaluation of understory plant response to logging: a study of redwood forests. Plant Ecology 194:179–194.

MADEJ, M. A. 2010. Redwoods, restoration, and implications for carbon budgets. Geomorphology 116:264–273.

———. 2011. Temporal and spatial distribution of landslides in the Redwood Creek Basin, Northern California. Pp. 149–179 *in* C. N. Medley, G. Patterson, and M. J. Parker (eds.), Proceedings of fourth interagency conference on research in the watersheds. U.S. Geological Survey Science Investigations Report 2011–5169, Fairbanks, AK.

MATHER, P. M. 1976. Computational methods of multivariate analysis in physical geography. John Wiley and Sons Press, London, UK.

MCCUNE, B. AND J. B. GRACE. 2002. Analysis of ecological communities. MJM Software Design, Glenden Beach, OR.

——— AND M. J. MEFFORD. 2011. PC-ORD. Multivariate Analysis of Ecological Data. Version 6. MJM Software Design, Gleneden Beach, OR.

MICHELS, K. K. H. AND W. H. RUSSELL. 2012. A chronosequence of vegetation change following timber harvest in naturally recovering coast redwood (*Sequoia sempervirens*) forests. Pp 283–292 *in* R. B. Standiford, T. J. Weller, D. D. Piirto, and J. D. Stuart (eds.), Proceedings of coast redwood forests in a changing California: a symposium for scientists and managers.

U.S. Department of Agriculture, U.S. Forest Service, PSW-GTR-238, Albany.

Noss, R. F. 1999. The redwood forest: history, ecology, and conservation of the coast redwoods. Island Press, Washington D.C.

O'Dell, T. E. 1996. Silviculture in the redwood region: a historical perspective. Pp. 15–17 *in* J. LeBlanc (ed.), Proceedings of coast redwood forest ecology and management. University of California Cooperative Extension, Humboldt State University, Arcata, CA.

O'Hara, K. L., J. C. B. Nesmith, L. Leonard, and D. L. Porter. 2010. Restoration of old forest features in coast redwood forests using early-stage variable-density thinning. Restoration Ecology 18:125–135.

Rittiman, C. A., Jr. and T. Thorson. 2006. National Cooperative Soil Survey: Mendocino County, California. Website http://soildatamart.nrcs.usda.gov/Manuscripts/CA694/0/MendocinoWP_CA.pdf (accessed 5 March 2006).

Russell, W. 2009. The influence of timber harvest on the structure and composition of riparian forests in the coastal redwood region. Forest Ecology and Management 257:1427–1433.

——— and C. Jones. 2001. The effects of timber harvesting on the structure and composition of adjacent old-growth coast redwood forest, California, U.S.A. Landscape Ecology 16:731–741.

——— and K. H. Michels. 2010. Stand development on a 127-yr chronosequence of naturally regenerating *Sequoia sempervirens* (Taxodiaceae) forests. Madroño 57:229–241.

——— and S. Woolhouse. 2012. 'Pygmy' old-growth redwood characteristics on an edaphic ecotone in Mendocino County, California. Pp 313–321 *in* R. B. Standiford, T. J. Weller, D. D. Piirto, and J. D. Stuart (eds.), Proceedings of coast redwood forests in a changing California: a symposium for scientists and managers. U.S. Department of Agriculture, U.S. Forest Service, PSW-GTR-238, Albany, CA.

———, J. R. McBride, and K. Carnell. 2000. Edge effects and the effective size of old-growth coast redwood preserves. Pp. 128–136 *in* D. N. Cole and S. F. McCool (eds.), Proceedings of wilderness science in a time of change RMRS-P-000. U.S. Department of Agriculture, U.S. Forest Service, Ogden, UT.

———, J. Sinclair, and K. H. Michels. 2014. Restoration of coast redwood (*Sequoia sempervirens*) forests through natural recovery. Open Journal of Forestry 4:106–111.

Rutland R. 2002. Big river land use history: 1852–2001. California Department of Forestry and Fire Protection, Santa Rosa, CA.

Sawyer, J. O., S. Sillett, J. H. Popenoe, A. Labanca, T. Sholars, D. L. Largent, F. Euphrat, R. F. Noss, and R. Van Pelt. 2000. Characteristics of redwood forests. Pp. 39–79 *in* R. F. Noss (ed.), The redwood forest: history, ecology, and conservation of the coast redwoods. Island Press, Washington D.C.

Scheller, R. M. and D. J. Mladenoff. 2002. Understory species patterns and diversity in old-growth and managed northern hardwood forests. Ecological Applications 12:1329–1343.

Sillett, S. C. and R. Van Pelt. 2007. Trunk reiterations promote epiphytes and water storage in an old-growth redwood forest canopy. Ecological Monographs 77(3):335–359.

Spies, T. A. and J. F. Franklin. 1996. The diversity and maintenance of old-growth forests. Pp. 235–248 *in* R. Szaro and D. Johnston (eds.), Biodiversity in managed landscapes. Cambridge University Press, Cambridge, UK.

Stone, E. C. and R. B. Vasey. 1968. Preservation of coast redwood on alluvial flats. Science 159:157–161.

USDA, NRCS. 2012. United States Department of Agriculture Web Soil Survey. Website http://websoilsurvey.nrcs.usda.gov (accessed 15 January 2012).

van Mantgem, P. J. and J. D. Stuart. 2012. Structure and dynamics of an upland old-growth forest at Redwood National Park, California. Pp. 333–343 *in* R. B. Standiford, T. J. Weller, D. D. Piirto, and J. D. Stuart (eds.), Proceedings of coast redwood forests in a changing California: a symposium for scientists and managers. U.S. Department of Agriculture, U.S. Forest Service, PSW-GTR-238, Albany, CA.

Wimberly, M. C. 2002. Spatial simulation of historical landscape patterns in coast forests of the Pacific Northwest. Canadian Journal of Forest Research 32:1316–1328.

Wu, L. and X. Guo. 2006. Response of two coast redwood (*Sequoia sempervirens* Endl.) varieties to moderate levels of salt and boron spray measured by stress symptoms: implications for landscape irrigation using recycled water. Environmental and Experimental Botany 58:130–139.

RELATIONSHIPS, INFRATAXA, AND HYBRIDS OF *ROSA GYMNOCARPA* (ROSACEAE)

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Abstract

The relationships of *Rosa gymnocarpa* Nutt. in connection with historical sect. *Gymnocarpae* Crép. and recent publications on *Rosa* phylogeny are discussed, noting the relative isolation of the species within the genus. All synonyms and segregate taxa are addressed; a lectotype is designated for the species and other names as needed. Two subspecies are recognized: subsp. *gymnocarpa,* primarily west of the Cascade-Sierra axis, and subsp. *helleri* (Greene) Ertter & W. H. Lewis, occurring from southeastern British Columbia and northwestern Montana to the northern Sierra Nevada in California. Subspecies *gymnocarpa* is further divided into var. *gymnocarpa* and var. *serpentina* Ertter & W. H. Lewis. A key, range descriptions, and representative specimens are provided for infraspecific taxa. Extensive zones of intergradation occur where the ranges intersect, especially in the Klamath and Siskiyou ranges of northeastern California and adjacent Oregon; intermediates are also common in northern Idaho and adjacent states and provinces. Presumed hybrids with sympatric species are discussed, including some across ploidy levels. Nothospecies *R. ×henryana* W. H. Lewis is described for the hybrid of *R. gymnocarpa* and *R. nutkana* C. Presl; *R. ×collaris* Rydb. (pro sp.) is transferred to nothospecies status as the hybrid of *R. gymnocarpa* and *R. woodsii* Lindl.

Key Words: Hybrid, lectotype, nomenclature, *Rosa gymnocarpa,* subspecies, varieties.

Rosa gymnocarpa Nutt. is among the most distinctive species in a genus that is notorious for a confusing combination of high phenotypic plasticity and interspecific hybridization (Lewis et al. 2015 [2014]). The species is particularly distinctive in its mature fruit, from which the sepals collectively and cleanly dehisce, giving rise to the specific epithet. In addition, achenes tend to be fewer, larger, and less hairy than sympatric species. Flowering material is also distinctive in its extremely small hips, solitary to few flowers on pedicels that are commonly and conspicuously stipitate-glandular, and doubly glandular-toothed leaflets with both surfaces completely glabrous. This is also one of the few roses that flourishes in partial shade, occurring in well-forested areas from British Columbia and Montana south in the mountains to southern California, with disjunct populations in the mountains of San Diego County. *Rosa gymnocarpa* evidently completely skirts the Great Basin, with the possible exception of the type of *R. leucopsis* Greene, purportedly from the midst of central Oregon's sagebrush steppe that is otherwise devoid of the species (Cronquist and Holmgren 1997).

Section *Gymnocarpae* and Molecular Analysis

Crépin (1876, p. 82) was sufficiently impressed with the distinctive characteristics of *Rosa gymno-* *carpa* to use it as the basis for a new section *Gymnocarpae* Crép. He did not maintain this section in subsequent publications (Crépin 1889, 1896), but instead included *R. gymnocarpa* within sect. *Cinnamomeae* (DC.) Ser. (=sect. *Rosa*). Crépin (1896) also believed that the diagnostic sepal articulation was shared by two Asiatic species, *R. beggeriana* Schrenk and *R. alberti* Regel. Rydberg (1917, 1918) retained *Gymnocarpae* among his "groups" of *Rosa*, expanding it to include *R. bridgesii* Crép. ex Rydb. and several names currently treated as synonyms of *R. gymnocarpa*. Boulenger (1936, 1937) used *Gymnocarpae* as a "Groupe" within sect. *Eglanteriae*, parallel to several other groups including a combined *Cinnamomeae-Caninae*. In addition to the species previously noted by Crépin as having comparable sepal articulation, Boulenger added three other Asiatic species: *R. willmottiae* Hemsl., *R. fargesiana* Boulenger, and *R. kotschyana* Boiss. The combination *R. gymnocarpa* var. *willmottiae* (Hemsl.) P. V. Heath has even been proposed, but with no more justification than that *R. willmottiae* "is too similar to *R. gymnocarpa* to be regarded specifically distinct" (Heath 1992). In reality, the two species have significant differences in armature, and the sepals of *R. willmottiae* are more tardily dehiscent.

Molecular analysis (Bruneau et al. 2007; Fougère-Danezan et al. 2015) does not support the association of the species suggested by Crépin, Rydberg, or

Boulenger as close relatives of *Rosa gymnocarpa*. Instead, *Rosa gymnocarpa* occurs in a well-supported clade with *R. pinetorum* A. Heller and one sample of *R. californica* Cham. & Schltdl. in Bruneau et al. (2007), probably indicating some gene flow among the source material (see discussion of hybrids with *R. californica* and *R. spithamea* S. Watson). Intriguingly, both *R. bridgesii* (and its glandular expression *R. lesterae* Eastw.) and *R. spithamea* (and its variant *R. granulata* Greene) fell into a separate well-supported clade with several Asian species. As for the other species proposed as relatives of *R. gymnocarpa*, *R. willmottiae* and *R. alberti* are in a third clade (further undermining Heath's proposal to treat the former as a variety of *R. gymnocarpa*), while *R. beggeriana* is in yet another clade. Although molecular phylogeny accordingly excludes a broadly defined *Gymnocarpae* from further consideration, it is worth noting that *R. gymnocarpa* itself is isolated on a separate "thumb" of the GADPH-based network produced by Fougère-Danezan et al. (2015). Some additional results beyond those specifically addressed in these two papers, which were focused on large-scale relationships within the genus, can be gleaned from the figured phylogenetic trees, in particular those involving the placement of samples provided by the senior author to Bruneau; these samples and results are discussed as appropriate in the current paper.

VARIATION WITHIN *ROSA GYMNOCARPA*

Within *Rosa gymnocarpa*, variation throughout the species' range has tended to be overlooked, except for early efforts by Greene (1912) that obfuscated rather than clarified any geographic patterns. Most of the 22 segregates and allies proposed by Greene were subsumed by Rydberg (1917, 1918) and subsequent authors. Erlanson (1930) mentioned the extensive variation within *R. gymnocarpa* but noted that "all attempts at splitting it into many species have failed".

In conjunction with the preparation of treatments of *Rosa* for California (Ertter 2012) and *Flora of North America North of Mexico* (Lewis et al. 2015), we have jointly or individually examined the complete or representative holdings of *Rosa gymnocarpa* from CAS/DS, CIC, MO, MONTU, OSC/ORE/WILLU, SRP, UBC, UC/JEPS, and WTU, supplemented with selected specimens from BM, GH, K, NY, RM, RSA, US, and WS, among other herbaria. We have also made extensive field observations and collections from throughout the range of *R. gymnocarpa*, allowing us to evaluate phenotypic variation within an ecogeographic setting and to determine possible hybrids based on co-occurring congeners.

Our studies have previously resulted in the formal recognition of one localized variant, var. *serpentina* Ertter & W. H. Lewis, restricted to ultramafic substrates in the Siskiyou Mountains of southwestern Oregon and northwestern California (Ertter and Lewis 2008). Comparable evaluation of variation beyond California was not completed in time for incorporation in our treatment of the genus for *Flora of North America North of Mexico* (Lewis et al. 2015), but ongoing research has subsequently supported the division of *Rosa gymnocarpa* into two subspecies, presented here. The rationale for our use of infraspecific ranks in *Rosa* (i.e., subspecies for components of a species that occupy significant geographic ranges, variety for more localized population clusters) is more fully addressed elsewhere (Lewis and Ertter 2007).

The first subspecies, which occurs primarily west of the Cascade-Sierra axis and includes the lectotype (designated below), tends to have a straight dominant axis, numerous prickles, and comparatively smaller and more numerous leaflets (Fig. 1A). In contrast, the second, more interior subspecies tends to be openly branched, with prickles often absent and with comparatively larger, fewer leaflets (Fig. 1B). As is characteristic for subspecies in other species of *Rosa* (Lewis and Ertter 2007, 2010), in which hybridization and phenotypic plasticity are well established, these are generalized patterns with an ecogeographic underpinning rather than sharply delimited, invariant distinctions. Subspecies differentiation furthermore breaks down in major transition zones associated with corridors across the Cascade-Sierra axis; i.e., the lower Fraser River in southwestern British Columbia, the Columbia River on the Washington-Oregon border, and in the Siskiyou and Klamath Mountains on the Oregon-California border, extending east to Modoc County, CA. Populations most closely representing the coastal rather than the interior subspecies can be found nearly to the Canadian border along the Columbia River in Washington; intermediates are common in northern Idaho, with sporadic occurrences also in Montana and southeastern British Columbia.

Variety *serpentina* occurs in the transition zone between the two subspecies in northwestern California and adjacent Oregon, and unsurprisingly combines features of the two subspecies: e.g., the branching pattern of the coastal subspecies and the leaflet number of the interior subspecies. In that we have settled on branching pattern as a most reliable and least ambiguous key character between the two subspecies, var. *serpentina* is here placed within the coastal subspecies.

Varieties previously proposed but rejected by us include *Rosa gymnocarpa* var. *pubescens* S. Watson, which is a synonym of *R. bridgesii,* and *R. gymnocarpa* var. *pinetorum* (A. Heller) Jeps., which is treated by us as *R. pinetorum*. As noted above, *R. gymnocarpa* var. *willmottiae* is an unsupported association of two unrelated species.

In the taxonomic treatment below, representative specimens are cited for all counties and provinces where assignment of the specimens among subspecies and variety was relatively certain. *Rosa gymnocarpa* occurs in additional counties (e.g., in interior Washington and northern Idaho) that are not cited if the vouchers were intermediate or insufficient for deter-
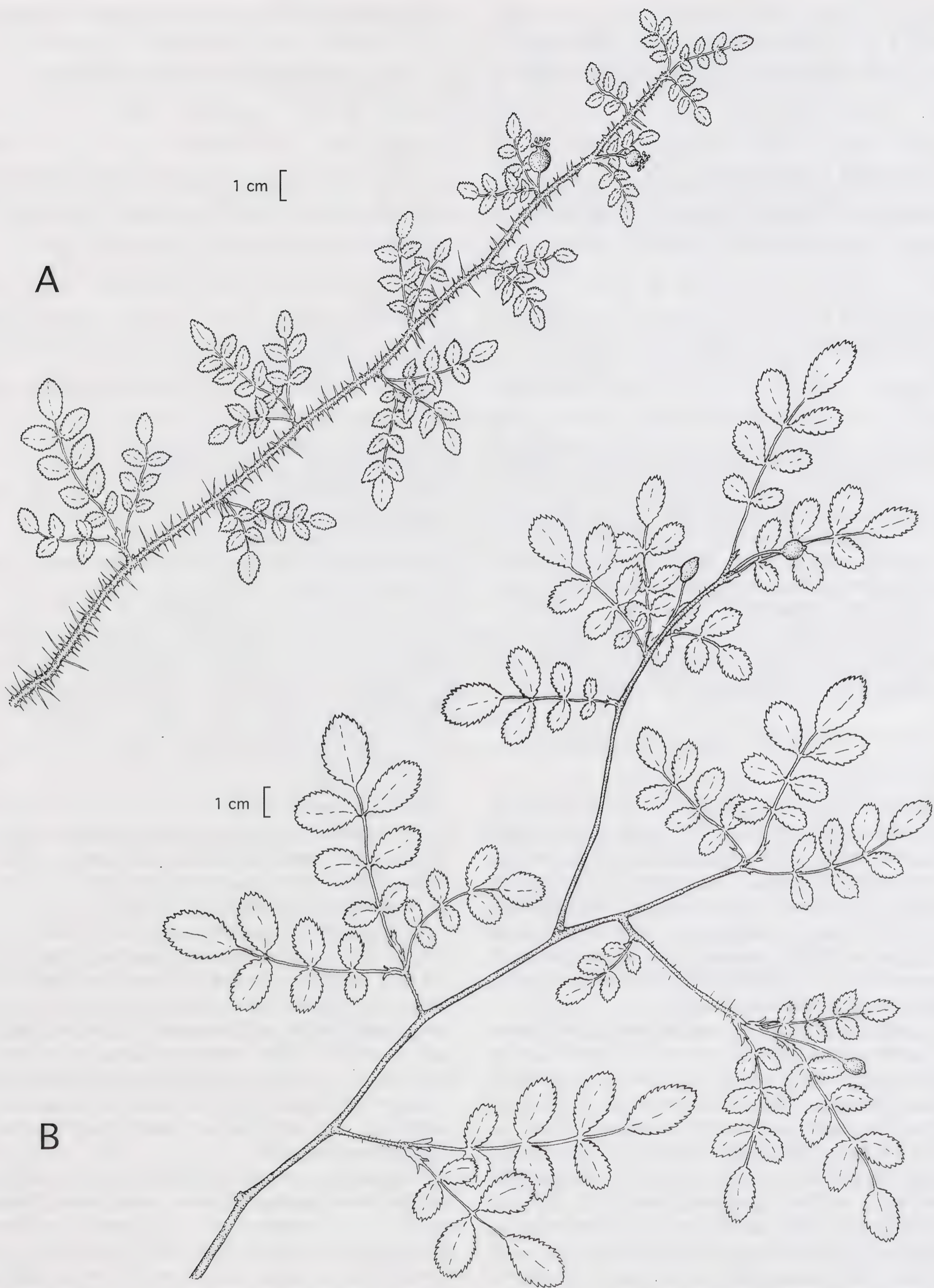
Fig. 1. Contrasting habits of subspecies of *Rosa gymnocarpa*. A. Extreme form of subsp. *gymnocarpa,* showing unbranched straight axis with fascicled leaves on short lateral spurs, abundant prickles, and relatively small leaflets (based on *Ertter 19020*, Alameda Co., CA). B. Extreme form of subsp. *helleri,* showing openly branched stem architecture, sparse or absent prickles, and relatively large leaflets (based on *Ertter 19304*, Shoshone Co., ID).

mination to subspecies. Cited specimens are supplemented with duplicates from *Consortium of Pacific Northwest Herbaria* (http://www.pnwherbaria.org/index.php) and *Consortium of California Herbaria* (http://ucjeps.berkeley.edu/consortium/). Non-imaged collections and other reports from counties beyond the cited distribution are problematic, especially given the frequency with which roses are misidentified. Duplicates of some Ertter collections have not yet been distributed; sets will be preferentially deposited in MO, UC, and regionally appropriate herbaria. Only the most basic label data is provided here (e.g., collector's last name; date only if lacking collection number), since further data is readily available from websites. Added information beyond that present on the label is in square brackets.

TAXONOMIC TREATMENT OF *ROSA GYMNOCARPA*

Rosa gymnocarpa Nutt., Flora of North America (Torr. & A. Gray) 1(3): 461. 1840. —TYPE: USA, Oregon [or possibly Washington], "Oregon woods", 1834 or 1835, *Nuttall s.n.* (lectotype, designated here: BM 602055/1024162, upper right specimen [Fig. 2]).

Rosa abietorum Greene, Leaflets of Botanical Observation and Criticism 2: 257. 1912. —TYPE: USA, Oregon, Klamath Co., Lake of the Woods, 25 Jul 1897, *Coville & Applegate 145* (holotype: US 380319; isotype: NY).

Rosa amplifolia Greene, Leaflets of Botanical Observation and Criticism 2: 258. 1912. —TYPE: USA, Oregon, Jackson Co., margin of Fish Lake, 18 Jun 1898, *Applegate [2500]* (holotype: US 381523).

Rosa glaucodermis Greene, Leaflets of Botanical Observation and Criticism 2: 255. 1912. —TYPE: USA, California: Siskiyou ("Shasta") Co., Shasta Springs, 9 Aug 1894, *Jepson 13890* (holotype: US 480045; isotypes: JEPS, POM).

Rosa gymnocarpa was first collected by David Douglas in British Columbia in 1824 or 1825, and afterwards in Oregon by Thomas Nuttall, who wrote the description incorporated in Torrey and Gray's two-volume *Flora of North America*. Lectotypification is needed not only because both collectors are cited in the protologue ("Oregon, in shady woods, common, *Nuttall! Douglas!*"), but also because the variation (e.g., some with flowers, other with mature fruit) among the specimens collected by Nuttall indicates that he collected the species more than once during his time in the Oregon Territory (which included the current state of Washington). He could have encountered subsp. *helleri* first when the Wyeth expedition crossed the Blue Mountains of northeastern Oregon in late August of 1834 (McKelvey 1955). From there, the party went north to Fort Walla Walla (present-day Wallula, Washington), before following the Columbia River to Fort Vancouver near the river's mouth. After spending the winter months in the Hawaiian Islands, Nuttall returned to Oregon, where he collected plants until proceeding to California by

FIG. 2. Nuttall collection of *Rosa gymnocarpa* in BM; the specimen in the upper right corner is the designated lectotype. The specimen to the immediate left is probably a continuation of the same stem, but at least some other specimens were most likely collected at other sites. Image reproduced with kind permission from the Natural History Museum (BM), London, UK.

way of Hawaii in late 1835. During this long period in western Oregon he would have encountered an abundance of subsp. *gymnocarpa,* and possibly intergrades between the subspecies as well.

First-stage lectotypification on Nuttall (vs. Douglas) has already been effected, by Piper (1906, p. 334) if not earlier. We hereby complete the process by designating the specimen in the upper right corner of BM sheet 602055/1024162 as the lectotype (Fig. 2). This particular element has mature fruit attached; the specimen to the immediate left is very likely a continuation of the same stem. The lower two specimens are clearly different, with large leaves and more open branching; one is in full flower and the other is at an intermediate developmental stage. The upper left specimen is a sterile shoot. There are two labels on this sheet: the usual label in Nuttall's hand in the lower left corner ("Columbia woods") and a separate chit ("Oregon woods. Nuttall's Herb.", also written on the back of the sheet) in a different hand below the lectotype. It is possible that both labels were intended for all five specimens on the sheet, which Nuttall had intentionally assembled to document variation within his new species. Even if

not, the one marked specifically as once being part of Nuttall's personal herbarium is an appropriate choice for lectotype. More importantly, the designated lectotype displays the majority of diagnostic features from the protologue (i.e., slender straight prickles, solitary flowers, naked fruit) and is a good exemplar of one of the subspecies (i.e., the coastal form) rather than being ambiguous in this regard. The coastal form accordingly becomes the autonym as a result of this lectotypification, while the interior form becomes subsp. *helleri* (below).

Given the probability of multiple gatherings by Nuttall, all other collections of *Rosa gymnocarpa* by Nuttall (and Douglas) should conservatively be treated as syntypes rather than isolectotypes. This includes the other elements on the BM sheet (with the probable exception of the specimen to the immediate left of the lectotype) and specimens with the handwritten Nuttall label in CGE, GH, K, NY, and PH. The GH specimen (HUH 32576) was provisionally annotated by Lewis as lectotype, but it is labeled as having been acquired from E. Durand well after the species was published. Those specimens with larger leaves and relatively open branching might represent subsp. *helleri* rather than subsp. *gymnocarpa*, but in the absence of more specific locality information they could simply be intermediates, or the occasional anomalous plants of subsp. *gymnocarpa*.

The types of synonyms cited above under *Rosa gymnocarpa* are all from the transition zone and cannot be unequivocally assigned to either subsp. *gymnocarpa* or subsp. *helleri*. This includes *R. abietorum* Greene, which is representative of the common form of *R. gymnocarpa* occurring in the divide across the Cascade Range and northern Siskiyou Mountains in southern Oregon and adjacent California. These tend to be relatively low-growing, diffuse-stemmed plants with plentiful slender prickles. Stem architecture and armature are closest to that of subsp. *gymnocarpa*, but with longer than average lateral flowering shoots; leaflet number and shape span the variation between subspecies. Populations in this area are also noteworthy in having a high percentage of pedicels with exceptionally short, sparse, or absent stalked glands, a condition only infrequently encountered elsewhere in the species' range.

The subspecies and varieties are distinguished using the following key, with the caveat that numerous collections from transitional regions cannot be unequivocally assigned to a named subspecies or variety. Length of lateral flowering shoots is measured from bud scar on the current season's growth to base of pedicel; care is sometimes needed to distinguish an unbranched axis representing more than 1 year's growth. Atypically long flowering shoots, and also more and/or longer prickles than otherwise typical, can sometimes be a response to browsing or other injury to the main axis. Branch angle includes that of lateral flowering shoots. Other non-diagnostic characters are as given in Ertter and Lewis (2008) and Lewis et al. (2015). Some intermediate collections from northern California occur at higher elevations than those given in the key below for unequivocal var. *gymnocarpa* or subsp. *helleri*, providing an upper elevational limit of 2200 m for the species excluding var. *serpentina*.

KEY TO INFRASPECIFIC TAXA OF *ROSA GYMNOCARPA*

1. Lateral flowering shoots usually several on straight unbranched axis of simple stem architecture, 0.3–7(–17) cm (sometimes a short spur with fascicled leaves, especially near coast); branch angle (35–)40–70(–80)°; prickles 1–8(–10) mm, most often numerous, sometimes sparse, rarely absent, infrastipular pairs often prominent (especially near coast); lateral leaflets 2–4 pairs, terminal leaflet (0.5–)1–3(–4) cm long; petals 7–12 mm long; mostly west of Cascade-Sierra axis, also scattered along Columbia River . . . . subsp. *gymnocarpa*
    2. Lateral leaflets commonly (2–)3–4 pairs, terminal leaflet elliptic to obovate or ovate, apically ± obtuse or sometimes nearly acute or rounded, (0.5–)1–3(–6) cm long; pedicels (1–)1.5–2.5(–3.5) cm long; plants to 1.5(–2.5) m tall; generally in partial shade in and at the edges of forests on non-ultramafic substrates; range of subspecies, 3–1300(–1800) m, flowering (February) April–July . . . . var. *gymnocarpa*
    2'. Lateral leaflets commonly 2 pairs, terminal leaflet broadly elliptic to nearly round, apically broadly obtuse to rounded, sometimes nearly truncate, 0.4–2 cm long; pedicels 1–1.5 cm long; plants to 0.6(–1.3) m tall; full sun in ultramafic chaparral and dwarf forests; Siskiyou Mountains of southwestern Oregon and northwestern California, 150–1500(–2300) m, flowering April–June. . . . . var. *serpentina*
1'. Lateral flowering shoots usually on separate axes of openly branched stem architecture, (1–)2–9 cm; branch angle 20–55(–65)°; prickles 1–5(–8) mm, most often sparse to absent, sometimes abundant, infrastipular pairs rarely prominent (except in Sierra Nevada); lateral leaflets 2–3(–4) pairs, terminal leaflet (1–)1.5–4.5 cm long; petals 10–14 mm long; mostly east of Cascade-Sierra axis, generally in partial shade in and at the edges of forests on non-ultramafic substrates, (120–)900–1800 m, flowering May–August . . . . subsp. *helleri*

1. Rosa gymnocarpa Nutt. subsp. *gymnocarpa*

1a. Rosa gymnocarpa Nutt. var. *gymnocarpa*

Rosa apiculata Greene, Leaflets of Botanical Observation and Criticism 2: 259. 1912. *R. gymnocarpa* f. *apiculata* (Greene) J. K. Henry, Flora of Southern British Columbia and Vancouver Island 174. 1915. —TYPE: USA, Washington, Island Co., Whidbey Island, Puget Sound, near Coupeville, Jul 1899, *Saunders s.n.* (holotype: US 364810; isotype: NY [fragment]).

Rosa gymnocarpa f. *lanceolata* J. K. Henry, Flora of Southern British Columbia and Vancouver Island 174. 1915. —TYPE: CANADA, British Columbia, Elgin [date and collector not indicated] (holotype: to be located and/or designated).

Rosa piscatoria Greene, Leaflets of Botanical Observation and Criticism 2: 256. 1912. —TYPE: USA, California: San Mateo Co., Iverson's Place, Pescadero Creek, May 1903, *Elmer [4923]*. (lectotype, designated here): US 665810; isolectotypes: CAS, MO, NY, POM, UC).

Rosa prionota Greene, Leaflets of Botanical Observation and Criticism 2: 256. 1912. —TYPE: USA, California: Lake Co., foothills S of Mt. Sanhedrin, midway between Potter Valley and Hullville, 14 Jul 1902, *Heller 5858* (holotype: US416864; isotypes: DS, GH, MO, NY, POM, RM).

The extreme form of autonymic *Rosa gymnocarpa,* which is most commonly encountered along the immediate coast from British Columbia to central California, has numerous short flowering shoots arising from the straight axis of the previous year's growth, with leaves often appearing nearly fascicled on short shoots (Fig. 1A). Leaflets tend to be relatively small (often less than 1 cm) and numerous; prickles are usually abundant, with the infrastipular pair often prominent and larger than the others. This last character is particularly notable in the types of *R. piscatoria* and *R. abietorum*; the former is sympatric with the "*orthacantha*" phase of *R. californica*, which might represent introgression with *R. gymnocarpa* (Lewis et al. 2015). Very small leaflets characterize the type of *R. prionota*, which is furthermore distinctive in having finely puberulent rachises and/or young stems. This puberulent condition also occurs in scattered collections from central California, without any further ecogeographic correlation. Forma *lanceolata* J.K. Henry is available for plants with unusually elongate hips, a condition more common in var. *serpentina*.

As documented by the representative specimens listed below, *Rosa gymnocarpa* var. *gymnocarpa* occurs from southwestern British Columbia south to Palomar Mountain in San Diego Co., CA, primarily west of the Cascade-Sierra axis. Only counties for which vouchers have been confirmed are listed, but the variety undoubtedly occurs in other coastal Oregon and Washington counties. Plants conforming to the diagnostic characters occur along the Columbia River at least as far inland as Stevens County, WA, well within what is otherwise the range of subsp. *helleri*, with intermediates common in northern Idaho and northwestern Montana. Intermediates are also frequent away from the immediate coast in British Columbia and along the Cascade Range in Washington and Oregon, and become the common form in the Siskiyou and Klamath mountains of northern California (where intergrades with var. *serpentina* and hybrids with *R. pisocarpa* A. Gray create further complications) and

Modoc County, CA. Variety *gymnocarpa* is the only representative of the species recognized as continuing south in the Coast Ranges of California to San Luis Obispo County, although occasional leggy collections approach subsp. *helleri* in branching architecture. The few unequivocal occurrences of this variety at the west base of the northern Sierra Nevada (i.e., Amador and Yuba counties) may have taken advantage of major rivers to cross the Central Valley. One collection from Plumas County (4 mi N of Grays Flat, *Howell et al. 54191* [CAS]) might represent var. *gymnocarpa,* but most other collections from this county are assigned to subsp. *helleri* or hybrids with *R. bridgesii*.

Populations further south are evidently relictual in nature, with no evident distinctions from var. *gymnocarpa* elsewhere in its range. Documentation of *Rosa gymnocarpa* in the Transverse Ranges in only known from a single sterile collection (*Ewan 7865* [POM, RSA]); F. Boutin (pers. comm. 2007) also recalls seeing small plants growing in crevices of canyon walls in shaded portions of upper Santa Anita Canyon above Arcadia. The relict population on Palomar Mountain in San Diego County is better documented, with some evidence of introgression with *Rosa californica* (e.g., *Ertter 15632* p.p. [UC]) or otherwise anomalous (e.g., *Jepson & Hall s.n.* [UC], which has exceptionally large infrastipular prickles and stipitate-glandular hips). In addition, F. Boutin (ibid.) reports noticing a small colony south of Julian but did not make a voucher; the locality is purported until documented.

Representative specimens. CANADA, BRITISH COLUMBIA. Sidney, Vancouver Island, *Lewis 1557* (MO, UBC); NE of Bella Coola, *McCabe 1440* (UC); Manning Park, *Beamish & Stone 7713* (UBC). USA, CALIFORNIA. **Alameda Co.**: Albany Hill, *Lewis & Ertter 15908* (MO); **Amador Co.**: Pine Grove, *Hansen 8986* (MO); **Colusa Co.**: Old Mill Camp, Trough Springs Ridge, *Sharsmith 4405* (UC); **Contra Costa Co.**: Alamo Canyon, Mt. Diablo, *Bowerman 895* (UC); **Del Norte Co.**: McNaught Place, Smith River, 1 Aug 1933, *Parks s.n.* (UC); **Glenn Co.**: near Bennet Spring on Newville-Covello Road, *Heller 11937* (CAS, DS, GH, MO, UC); **Humboldt Co.**: Redwood Creek between Blue Lake and Willow Creek, *Wiggins 4695* (CAS, DS, GH, POM, UC), Grasshopper Peak, Bull Creek State Park, *Tracy 19267* (UC); **Lake Co.**: S of Mount Sanhedrin, *Heller 5858* (TYPE of *R. prionata*: DS, GH, MO, NY, POM, US); **Los Angeles Co.**: N slope Monrovia Peak, San Gabriel Mountains, *Ewan 7865* (RSA); **Marin Co.**: Lagunitas, *Parish 19061* (UC); **Mendocino Co.**: Mendocino-Comptche Road, *Ertter 15652* (MO, UC); **Monterey Co.**: Del Monte Forest between S. F. B. Morse Botanical Reserve and 5th Gate, 21 Jun 1992, *Yadon s.n.* (JEPS), road to Cone Peak, *Ertter 14901* (MO, UC); **Napa Co.**: 5 mi S of Calistoga, *Heller 13838* (DS, MO, NY); **San Diego Co.**: Palomar Mountain, *Ertter 15631* (MO, UC); **San Francisco Co.**: Mt.

Davidson, *Raven 9006* (CAS); **San Luis Obispo Co.**: ridge NW of Cuesta Pass, *Hoover 9408* (CAS, OBI, UC); **San Mateo Co.**: Pescadero, *Elmer 4923* (TYPE of *R. piscatoria*: CAS, MO, NY, POM, UC, US); **Santa Clara Co.**: Coyote Creek, *Dudley 4098* (DS, GH, UC); **Santa Cruz Co.**: Saratoga, *Pendleton 57* (UC); **Shasta Co.**: 0.5 mi SE of Sims Flat Campground, *Taylor 2823* (MO); **Sonoma Co.**: Kenwood, Sonoma Canyon, *Jepson 10013* (JEPS); **Tehama Co.**: between Paynes and Cohasset on Ponderosa Way, *Oswald & Ahart 8441* (CHSC, JEPS); **Trinity Co.**: summit on rd from Douglas City to Hayfork, *Howell 30351* (CAS); **Yuba Co.**: 3 mi SE of Challenge, *Ahart 9051* (CHSC, JEPS). OREGON. **Benton Co.**: Mary's Peak, *Merkle 40-178* (OSC); **Clackamas Co.**: Oregon City, *Lewis 1510* (MO); **Clatsop Co.**: Onion Peak, *Chambers 3282* (OSC); **Columbia Co.**: 0.25 mi E of Rainier on Hwy 30, *Hanneman 38* (SRP); **Coos Co.**: Golden & Silver Falls State Park, *Thompson & Skeese 88-1433* (ORE in OSC); **Curry Co.**: Winchuck Campground S of Brookings, *Ireland 2745* (ORE in OSC); **Deschutes Co.**: McKenzie Pass, *Benson 2248* (MO); **Douglas Co.**: Myrtle Island in Umpqua River, *Thompson & Thompson 5831* (ORE in OSC); **Hood River Co.**: Dry Creek Falls, Cascade Locks, *Gustafson 189* (OSC); **Jackson Co.**: Beaver Creek Road to Dutchmans Peak, *Ertter 17491* (MO, UC); **Josephine Co.**: Oregon Caves, *Leach 1880* (ORE in OSC); **Lane Co.**: Wendling, *Mason 8663* (ORE in OSC, WS); **Linn Co.**: ca 5 mi SE of Idanha, *Halse 4537* (NY, OSC); **Marion Co.**: N Breitenbrush Road 1/4 mi from guard station, *Detling 5683* (ORE in OSC); **Multnomah Co.**: mountains above Bridal Veil, *Peck 2155* (WILLU in OSC); **Tillamook Co.**: 3 mi SE of Blue Lake, *Chambers 4114* (OSC); **Wasco Co.**: Hwy 50 near junction of Oregon Skyline Road, *Hitchcock & Martin 4795* (MO, NY, UC, WS, WTU); **Washington Co.**: Forest Grove, *Lloyd 91* (NY); **Wheeler Co.**: 2.5 mi S of summit on Prineville-Mitchell Road, *Eggleston 22105* (MO); **Yamhill Co.**: ca 11.5 mi W of Carlton, *Halse 3223* (CAS, OSC, WS, WTU). WASHINGTON. **Clallam Co.**: Mount Angeles, 22 Jul 1929, *Rigg s.n.* (WTU); **Clark Co.**: Green Mountain Resort Conservation Easement, *Habegger 365* (WTU); **Cowlitz Co.**: Kelso, *Lewis 1506* (MO); **Grays Harbor (formerly Chehalis) Co.**: near Montesano, *Heller & Heller 3897* (MO, NY, UC, WS); **Island Co.**: Whidbey Island, near Coupeville, Jul 1899, *Saunders s.n.* (TYPE of *R. apiculata*: US); **Jefferson Co.**: Hoh River, *Murie 1143* (MO); **King Co.**: Fort Lewis, *Lewis 1500* (MO), Rattlesnake Ledge, Cedar River Watershed, *Gage et al. 7207* (WTU); **Kittitas Co.**: E entrance Stampede Pass Tunnel, *Gage & Rodman 306* (WTU); **Lewis Co.**: Toledo, *Lewis 1505* (MO); **Mason Co.**: Dayton Cutoff Road, *Freer 201* (WTU); **Pierce Co.**: 6 mi W of Gig Harbor, *Lewis & Lewis 21156* (MO); **San Juan Co.**: Friday Harbor, *Blanchard 100* (UC, WTU); **Skagit Co.**: 5 mi S of Anacortes, *Hitchcock 3479* (CAS, DS, UC, WS, WTU); **Skamania Co.**: Prindle, *Suksdorf 11694* (WS, WTU); **Snohomish Co.**: Mount

Dickerman, *Thompson 14733* (CAS, DS, UC, WS, WTU); **Stevens Co.**: Columbia River 10 mi below Northport, *Rogers 552* ("Ferry Co." on label: CAS, DS, ORE in OSC, WS, WTU); **Thurston Co.**: Bucoda, *Lewis 1502* (MO); **Whatcom Co.**: Glacier, *Eggleston 21729* (NY).

1b. ***Rosa gymnocarpa*** Nutt. var. ***serpentina*** Ertter & W. H. Lewis, Madroño 55: 174. 2008. —TYPE: USA, California, Del Norte Co., Old Gasquet Toll Road 1.3 mi from junction of North Fork Rd., *Ertter 12768* (holotype: UC; isotypes: MO, NY, OSC).

As noted previously, var. *serpentina* is placed here within subsp. *gymnocarpa* primarily on the basis of stem architecture and armature, but leaflet number and shape are more comparable to those of subsp. *helleri*. It differs from both var. *gymnocarpa* and subsp. *helleri* in being consistently shorter on average, having somewhat more leathery leaves, and occurring in full sun on ultramafic substrates centered in the Siskiyou Mountains of northwestern California and southwestern Oregon.

Addenda to the vouchers of var. *serpentina* cited in the protologue (Ertter and Lewis 2008) consist primarily of collections (including by the senior author) from the extensive ultramafics near Gasquet in Del Norte County, CA, where the variety is most abundant. Additional collections are also now confirmed from the Preston Peak area (*Kildale 965* [DS]), *Wheeler 8341* [(MO, RSA]) in Siskiyou County, CA, and from Snow Camp Mountain (*Leach 2523* [ORE in OSC]) and Pearsoll Peak (*Gage 4740* [WTU]) in Curry County, OR. Witte's collections (*243, 748, 751* [HSC]) are probably further documentation of var. *serpentina* on Horse Mountain, Humboldt County, CA, although the specimens have not been confirmed by the authors. Addenda representing new localities are as follows:

Addenda to representative specimens (as previously cited in Ertter and Lewis 2008). USA, CALIFORNIA. **Tehama Co.**: 0.6 mi N of Tedoc Gap, *Ertter & Shevock 17081* (UC). OREGON. **Curry Co.**: W side Vulcan Peak, *Denton 3687* (WTU); **Josephine Co.**: 1 mi N of Serpentine Point, *Johanson & Johanson 27* (OSC, WTU), 15.7–17 mi W of Selma on Illinois River Rd, *Denton 2435* (WTU).

The range of var. *serpentina* might also occur further east and south, but interpretation of individual collections out of context becomes increasingly difficult within the range of subsp. *helleri* and intergrades between the subspecies. Both var. *serpentina* and subsp. *helleri* have similar leaflet shape and number, such that a depauperate specimen of the latter could be difficult to distinguish from the former in the absence of habitat and population data. The majority of candidates for possible inclusion within var. *serpentina*, pending further study, are in Siskiyou County, CA, southwest of Mount Shasta; examples include MacBride Spring Road (*Cooke 11094* [DS])

and Dunsmuir (*Eastwood 1321* [CAS]). Other candidates for inclusion are scattered elsewhere in Siskiyou (e.g., Cecilville, *Kildale 9126* [DS]) and Trinity (e.g., Coffee Creek, 16 Jun 1956, *McClintock s.n.* [CAS], Scorpion Lake, *Taylor 18160* [JEPS]) counties, CA, and in Jackson County (e.g., Siskiyou Summit, *Mason 4023* [UC]), OR. Some collections from the mountains of northeastern Mendocino County (e.g., Hulls Mountain Road near Monkey Rock, *Ertter 19051* [UC]), CA, also approach var. *serpentina*.

2. Rosa gymnocarpa Nutt. subsp. **helleri** (Greene) Ertter & W. H. Lewis, **stat. & comb. nov.**
Rosa helleri Greene, Leaflets of Botanical Observation and Criticism 2: 259. 1912. —TYPE: USA, Idaho, Nez Perce Co., Lake Waha, 25 Jun 1896, *Heller [3317]* (holotype: US 267361; isotypes: DS, UC [see discussion]).
Rosa dasypoda Greene, Leaflets of Botanical Observation and Criticism 2: 260. 1912. —TYPE: USA, Oregon, Wallowa Co., Bear Creek, 28 Aug 1897, *Sheldon [8815]* (holotype: US 528469; isotype: NY).
Rosa leucopsis Greene, Leaflets of Botanical Observation and Criticism 2: 258. 1912. —TYPE: USA, Oregon, Lake Co., sage plains [near Wagon Tire Mountain], 29 Sep 1896, *Brown 99* (holotype: US 283078; isotypes: MO, NY, RM, UC).

Subspecies *helleri* is the dominant expression of *Rosa gymnocarpa* in the interior part of the species' range, east of the Cascade Range divide and in the northern Sierra Nevada, with intergrades extending west to the Klamath Mountains in northern California. In general, plants of subsp. *helleri* have more open branching than subsp. *gymnocarpa*, with solitary flowers often terminal on two or more successive years' growth of a branched or unbranched lateral axis (Fig. 1B). Prickles are usually sparse, or even absent, except at the base of the plant and on new stems. Leaflets average larger and fewer in number than subsp. *gymnocarpa*, though with considerable overlap. Leaflet teeth are more likely to be crenate but can also be serrate, as is the more common condition in subsp. *gymnocarpa*.

The northeastern range of subsp. *helleri* includes southeastern British Columbia (at least as far north as Revelstoke) and northwestern Montana west of the Continental Divide; reports from east of the Divide are suspect until and unless determinations are confirmed. The subspecies is common in northern and central Idaho, though apparently intergrading with subsp. *gymnocarpa* at lower elevations. Documentation of the southern range in Idaho is spotty, further complicated by introgression with *Rosa woodsii* Lindl. subsp. *ultramontana* (S. Watson) Roy L. Taylor & MacBryde (i.e., the type of *R. collaris* Rydb.; see discussion under hybrids) and, for historical collections, the sequential creation of current counties from a handful of larger ones. Ada County (estab. 1864) originally included what is now Canyon (estab. 1891), Payette (estab. 1917), and southern Gem (estab. 1915) counties, while Washington County (estab. 1879) included what is now Adams (estab. 1911), northern Gem, and the southeasternmost corner of Idaho (estab. 1861) counties (Boone 1988). A 1911 collection from along the river in Boise (*Clark 320* [DS, MO, NY]) is probably in fact from within the bounds of modern-day Ada County, but recent floristic surveys by the senior author have failed to relocate any extant populations; the 1911 collection may have been a waif from upstream populations in the Boise Mountains, or from a peripheral population subsequently wiped out by dam building and other streamside alterations. The apparent absence from Custer County may represent lack of documentation. One purported voucher from Lemhi County (*Hitchcock & Muhlick 14252* [WS, WTU]) has been re-identified as *R. woodsii*.

To the west, subsp. *helleri* occurs in the mountains of northeastern Oregon and adjacent Washington, and along the east side of the Cascade Range where intergrades with subsp. *gymnocarpa* are common. The subspecies is also the primary form of *Rosa gymnocarpa* in the northern Sierra Nevada, extending south at least to El Dorado County, CA. Most collections of the species from Modoc County and the Klamath and Siskiyou mountains are intermediate between the subspecies, but at least some are assignable to subsp. *helleri*.

The sample of *Rosa gymnocarpa* included in the molecular analyses of Bruneau et al. (2007) and Fougère-Danezan et al. (2015) is of subsp. *helleri*, from the topotype locality in northern Idaho (*Ertter 18001* [MO]).

In addition to the DS and UC specimens cited as isotypes of *Rosa helleri*, there are several other collections that differ in either collection date (17 Jun [MO]; 22 Jun [RM]) or collection number (*3323* [NY, WTU]). Heller's collection number of *3317* is on the holotype at US though not specified in the protologue.

Representative specimens. CANADA, BRITISH COLUMBIA. Fernie, *Lewis 1298* (MO, UBC), 21.3 mi N of Revelstoke, *McCabe 5454* (UC), Botanie Valley, *Krajina 1958* (UBC). USA, CALIFORNIA. **Butte Co.:** French Creek ca 3 mi N of Sly Creek Dam, *Ahart 4464* (CAS, CHSC, MO); **El Dorado Co.:** 3 mi E of Camino, *Robbins 1079* (UC); **Modoc Co.:** Johnson Creek E of Hwy 299, *Bartholomew 6855* (CAS); **Nevada Co.:** 1 mi S of Skillman Flat Campground, *Ahart 9055A* (CHSC, JEPS); **Plumas Co.:** Butterfly Valley, *Ahart 9270* (CHSC, JEPS), ca 5 mi NW of La Porte, *Ahart 9764* (CHSC, JEPS, MO); **Shasta Co.:** 6.8 mi S of McCloud, *Almeda 7406* (CAS); **Sierra Co.:** ca 5.5 mi NE of Alleghany, *Ahart 9717* (CHSC, JEPS, MO); **Siskiyou Co.:** Black Bear Summit, *Alexander & Kellogg 5593* (DS, UC); **Tehama Co.:** ca 4 mi S of Deer Creek bridge on Hwy 32, *Ahart 9182* (CDA, CHSC, JEPS); **Yuba Co.:**

ca 1.5 NE of Strawberry Valley, *Ahart 10628* (CHSC, JEPS). IDAHO. **Adams Co.**: 8 mi N of Council, *Kramer N146* (ID); **Benewah Co.**: Plummer, *Christ 3837* (NY); **Boise Co.**: proposed Monumental Creek RNA, *Wellner 3888A* (ID); **Bonner Co.**: Priest Lake, *Nelson & Nelson 3035* (UC); **Boundary Co.**: near Copeland, *Ehlers & Erlanson 218* (MO); **Clearwater Co.**: 5 mi S of Orofino, *Baker 13980* (ID, WTU); **Elmore Co.**: Pine Grove, 19 Jun 1914, *Dunkle s.n.* (ID); **Idaho Co.**: Papoose Creek 8 mi SW of Riggins, *Davis 2361* (UC), Three Devils Creek 4 mi below Lowell, *Constance & Rollins 1609* (MO, WS); **Kootenai Co.**: Lake Coeur d'Alene, *Heller [Sandberg 619]* (MO, NY, ORE in OSC); **Latah Co.**: Moscow Mountains, *Abrams 662* (UC); **Nez Perce Co.**: about Lake Waha, *Heller 3317* (TYPE of *R. helleri*: DS, UC, US); **Shoshone Co.**: 5 mi SE of Wallace, *Ertter 19304* (MO, UC); **Valley Co.**: Ponderosa State Park, *Duft 1134* (CIC), trail to Bull Creek from Boiling Springs, *Smith 7848* (SRP); **Washington Co.**: Middle Fork Weiser River, *Jones 6279* (DS, MO, NY). MONTANA. **Flathead Co.**: Big Fork, *MacDougal 897* (NY), trail to Howe Lake, Glacier NP, *Yuncker & Yuncker 6765* (NY); **Lake Co.**: Yellow Bay, Flathead Lake, *Harvey 4980* (MONTU); **Lincoln Co.**: Kootenai River 60 mi W of Kalispell, *Hitchcock 17675* (WTU); **Mineral Co.**: Fish Creek Canyon near mouth of Cache Creek, *Hitchcock 1734* (MONT, MONTU); **Missoula Co.**: Holland Lake 80 mi NE of Missoula, *Cronquist 7878 p.p.* (NY [mixed with *R. acicularis*]), 11 mi SE of Missoula, *Stickney 745* (MONT, WTU); **Powell Co.**: Ovando, near camp above Pitkin Ranch, *Kirkwood 1175* (MO, MONTU, UC): **Ravalli Co.**: Lower Watchtower Creek Trail, *Lackschewitz 5876* (MONTU); **Sanders Co.**: 6 mi N of Thompson Falls, 2 Aug 1957, *Booth s.n.* (MONT). OREGON. **Baker Co.**: E Eagle Creek, *Head 932* (OSC); **Grant Co.**: Trout Pond campground, Strawberry Mountains, *Thompson 97017* (OSC); **Jackson Co.**: Huckleberry Mountain, Rte 60 at Mill Creek, *Ertter 17475* (MO, UC), Tub Spring, *Detling 4355* (OSC); **Jefferson Co.**: Abbot Butte Springs intersection, *Swedberg 77* (OSC); **Klamath Co.**: Swan Lake Valley, *Applegate 3191* (US), Yainax Ridge SW of Beatty, *Ertter 17682* (UC); **Linn Co.**: Hunt's Cove, Mount Jefferson area, *Leach 4558* (ORE in OSC); **Umatilla Co.**: ca 1.5 mi SW of Meacham, *Grimes et al. 1889* (CIC); **Union Co.**: 18 mi N of Elgin, *Peck 18341* (WILLU in OSC); **Wallowa Co.**: Bear Creek, *Sheldon 8815* (TYPE of *R. dasypoda*: NY, US); **Wasco Co.**: Eight Mile Creek, *Jones 4086* (CAS, MONTU, WTU). WASHINGTON. **Asotin Co.**: Blue Mountains, *Jones 985* (WS, WTU); **Chelan Co.**: Tronsen Creek 4 mi NW of Blewett Pass, *Ertter 21271* (MO); **Columbia Co.**: Wildcat Spring, *St. John 8314* (WS); **Ferry Co.**: ca 20 mi E of Republic, *Hitchcock 17571* (ID, UC, WS, WTU); **Kittitas Co.**: Peoh Point, South Cle Elum Ridge, *Jensen et al. 141* (WTU); **Okanogan Co.**: Twisp River Road, *Myers 245* (MO); **Spokane Co**: Newman Lake, 27 May 1938, *Stillinger s.n.* (ID); **Whitman Co.**: Kamiak

Butte, 20 Jul 1899, *Piper s.n.* (WS); **Yakima Co.**: E side of Mt. Adams, *Suksdorf 6836* (WS).

HYBRIDS BETWEEN *ROSA GYMNOCARPA* AND OTHER SPECIES

The morphological distinctiveness of *Rosa gymnocarpa* is evidently not matched by reproductive barriers with other species in sect. *Rosa*, even across ploidy levels. Obvious hybrids can be regularly found in areas of sympatry, recognized when diagnostic characters of *R. gymnocarpa* occur in combination with such features as hairy leaflets, more numerous achenes, and/or semi-persistent sepals. A synopsis of possible hybrids is provided below, with chromosome number (as given in Lewis et al. 2015) referring to the other parent; *R. gymnocarpa* is diploid ($2n = 14$). This synopsis complements a separate paper (Lewis in prep.) in which several new nothospecies in *Rosa* are described; those having *R. gymnocarpa* as one parent have been incorporated into the current paper.

× *Rosa acicularis* ($2n = 42$): Cronquist (1961, p. 166) suggested that the type of *R. collaris* was the hybrid of *R. gymnocarpa* with the hexaploid *R. acicularis* Lindl. As discussed below under *R. woodsii*, we believe *R. woodsii* subsp. *ultramontana* is the more likely second parent of the specimen in question. As a more plausible candidate for a hybrid involving *R. acicularis*, at least the WTU sheet of *Cronquist 7878* (W end Holland Lake, Missoula Co., MT) is a mixed collection of *R. acicularis* subsp. *sayi* (Schwein.) W. H. Lewis and its possible hybrid with *R. gymnocarpa* subsp. *helleri*. However, it is equally possible that the first parent is actually *R. woodsii*, which could easily have been present as well. Cronquist identified the entire gathering as *R. acicularis*, but noted on the label the presence of both large- and smaller-flowered forms, among other distinctions.

× *Rosa bridgesii* ($2n = 14, 28$): Putative hybrids, most likely with subsp. *hellerii*, are occasionally encountered in the northern Sierra Nevada and southern Cascade Range. Such plants can resemble *R. gymnocarpa* in gestalt, but have hairy leaves and/ or persistent sepals, or are more like *R. bridgesii* but with stalked-glandular pedicels. Examples include *Ahart et al. 9742* (CHSC, JEPS) from Butte County and *Ertter et al. 15686* (UC) from Plumas County, CA. The types of *R. covillei* Greene and *R. yainacensis* Greene, both from Klamath County, OR, might also represent this hybrid, but at present both are treated as unplaced synonyms (discussed below).

× *Rosa californica* ($2n = 28$). As addressed by Lewis et al. (2015), the "*orthacantha*" phase of *R. californica*, occurring in brushlands and open woodlands around Monterey and San Francisco bays, possibly results from introgression with var. *gymnocarpa*. As previously noted, the relict population on Palomar Mountain in San Diego County also shows

some evidence of introgression with *Rosa californica* (e.g., *Ertter 15632* p.p. [UC]). Root tips from at least one sample of "*orthacantha*" plants had $2n = 21$ chromosomes (D. Zlesak and W. H. Lewis, unpubl.), indicating crosses between species of different ploidy levels. Further evidence is provided by the otherwise anomalous clustering of one sample of *R. californica* with *R. gymnocarpa* on a distinct clade in Bruneau et al. (2007). This particular sample (*Ertter 17954*, Alameda Co., CA) was intended to represent "good" *R. californica*, growing at the base of a hill where both var. *gymnocarpa* and the "*orthacantha*" phase (*Ertter 17955*) occur This suggests that even the plants morphologically consistent with *R. californica* had introgression from *R. gymnocarpa* and also provides a possible alternate explanation for the dual occurrence of tetraploid *R. californica* on the Bruneau et al. (2007) phylogenetic trees. Although the type of *Rosa californica* var. *orthacantha* C. Presl has not been seen, from the description it is probably representative of this presumed hybrid. The type of *R. bolanderi* Greene might have the same parentage, or perhaps have *R. spithamea* as one parent instead.

Rosa bolanderi Greene, Leaflets of Botanical Observation and Criticism 2: 261. 1912, as *Rosa Bolandri*. —TYPE: USA, California: [Alameda Co.], Oakland Hills, *Bolander s.n.* (holotype: US 45934; isotype: NY). From the protologue, Greene thought this was probably "an unique specimen" that was "evidently not distributed". Although there is a duplicate at NY, the US specimen is here accepted as holotype (rather than lectotype).

Rosa californica [var.] β. *orthacantha* C. Presl, Epimeliae Botanicae 202. 1851. —TYPE: USA, California: [Monterey Co.], Monterey, *Haenke s.n.* (holotype: PR [not seen]).

× *Rosa nutkana* ($2n = 42$): Henry (1915) reported the hybrid of *R. gymnocarpa* [subsp. *gymnocarpa*] and *R. nutkana* C. Presl [subsp. *nutkana*] to be "abundant locally" at Crescent [Beach], in coastal southwestern British Columbia. Although his voucher (UBC V78778) was annotated by one of us (Lewis) as *R. nutkana* subsp. *macdougalii* (Holzinger) Piper, this subspecies does not occur on the coast, and Henry's reference to sterile pollen supports the hybrid hypothesis. Another collection from coastal southwestern British Columbia, 22 May 1914, *Taylor s.n.* from Savary Island (UBC V139598), is also a strong candidate for the same hybrid parentage. As noted in faint anonymous pencil on the sheet, the specimen combines the weak prickles of *R. gymnocarpa* and the large flowers of *R. nutkana* with evidently empty pollen grains.

Less evidence exists for possible hybrids of *R. gymnocarpa* subsp. *helleri* and *R. nutkana* subsp. *macdougalii*, in spite of the wide area of overlap. One candidate is *Ertter 19482* from near McCall in Valley Co., ID; although comparable to nearby *R. nutkana* subsp. *macdougalii* (e.g., *Ertter 19481*), glandular sepals and leaf margins suggest introgression from co-occurring *R. gymnocarpa* subsp. *helleri* (e.g., *Ertter 19483*).

In coordination with a separate paper (Lewis in prep.), the hybrid between *Rosa gymnocarpa* and *R. nutkana* is named here as a new nothospecies honoring Joseph Kaye Henry (1866–1930), author of *Flora of southern British Columbia and Vancouver Island* (Henry 1915) and the earliest botanist in British Columbia to discover and publish on *Rosa* hybridity.

Rosa ×*henryana* W. H. Lewis, **nothosp. nov.** [= *Rosa gymnocarpa* Nutt. × *Rosa nutkana* C. Presl]. — TYPE: CANADA, British Columbia, Vancouver, Crescent [Beach], 2 Jun 1915, *J. K. Henry s.n.* (holotype: UBC V78778).

Stems tall, slender, and vigorous with prickles dense below and slender above; leaflets oval, round at apex, mostly eglandular; pedicels glabrous to stipitate-glandular; flowers bright-pink, 4.5–5 cm broad; hips small and few, some stipitate-glandular. Coastal southwestern British Columbia, abundant locally.

Paratype: CANADA. British Columbia, Savary Island, 22 May 1914, *Taylor s. n.* (UBC V139598).

× *Rosa pisocarpa* (subsp. *pisocarpa*: $2n = 14$; subsp. *ahartii*: $2n = 28$): This was among the several putative hybrids listed by Rydberg (1918). Examples involving subsp. *pisocarpa* include *Ertter 17469* (MO, UC) from Jackson County, OR, and an apparent hybrid swarm documented by Tracy collections (e.g., *17889* [DS, UC], *18438½* [UC]) from Box Camp near Trinity Summit, Humboldt County, CA. Possible hybrids between *R. gymnocarpa* subsp. *helleri* and *R. pisocarpa* subsp. *ahartii* Ertter and Lewis include *Ahart 14067* (CHSC, JEPS) and *15800* (CHSC, JEPS, MO, RSA), both from Butte County, CA; such specimens often have the general appearance of *R. pisocarpa* subsp. *ahartii* but have glabrous leaves and atypically small hips in bud. *Heller 12203* (CAS, DS, MO, NY, UC) and *Oettinger 970* (HSC, RSA, UC) from Siskiyou County, CA, are also strong candidates for hybrids between these two species, but from the subspecies intergrade zone for both.

× *Rosa spithamea* ($2n = 14$): Further research is needed to determine whether collections keying to *R. pinetorum* from west-central California but from outside the range of this species as given by Ertter (2012) and Lewis et al. (2015) might actually represent hybrids between subsp. *gymnocarpa* and *R. spithamea* (e.g., *Ertter 19088* from Marin County). *Linsdale 222* (UC) from Monterey County, CA, and *Hutchinson 969* (JEPS) from Jackson County, OR, are also candidates for this hybrid parentage. Alternatively, it is possible that even "core" material on which the current circumscription of *R. pinetorum* is based derives from hybrids between *R. gymnocarpa* and *R. spithamea*, combining the eglandular hips of the former with the low stature and persistent sepals of the latter. Bruneau et al. (2007) associate *R. pinetorum* with *R. gymnocarpa* on

a well-supported clade, whereas *R. spithamea* falls in a different clade. The sample for *R. pinetorum* used in their study was taken from propagated material vouchered by *Ertter 11888*, which originated from a site in Monterey County where *R. gymnocarpa* (*Ertter 11887*) and possible *R. spithamea* (sterile; *Ertter 11886*) were also collected.

× **Rosa woodsii** ($2n = 14$): The most widespread hybrid, and that most studied by the authors (by virtue of a population occurring on property partly owned by the senior author), is that between *R. gymnocarpa* subsp. *helleri* and *R. woodsii* subsp. *ultramontana*. Rydberg (1918) was the first to propose this hybrid combination, using as the *woodsii* parent the synonyms *R. macounii* Greene, *R. pyrifera* Rydberg, and *R. woodsii* s.s. from western Montana. Plants identified as this hybrid occur where the ranges of the parental species overlap, from southeastern British Columbia to northeastern California, with the greatest concentration in central Idaho. These plants are not morphologically consistent, indicating their status as recurring hybrids; the leaf rachis is often puberulent, leaflets are sometimes hairy and/or sparsely or scarcely glandular-dentate, sepals are often persistent or tardily deciduous, and/or hips are larger than average for *R. gymnocarpa*. Material in cultivation has been confirmed diploid ($2n = 14$) by D. Zlesak and W. H. Lewis (unpubl.).

The type of *Rosa collaris* Rydb. is evidently representative of this hybrid, as is the type of *R. oligocarpa* Rydb. The former has priority and is accordingly adopted here as nothospecies *R.* ×*collaris* Rydb. (pro sp.). As written by E.W. Erlanson on the RM duplicate of *R. collaris* in 1926, "Note that specimen lined off [right-most] clearly belongs to the *Gymnocarpae*. Judging from this sheet *R. collaris* should be taken from the Cinnamomeae and placed beside *R. Bridgesii*". Her conclusion, and ours, differs from that of Cronquist (1961, p. 166), who suggested that *R. collaris* was a hybrid between *R. woodsii* and the hexaploid *R. acicularis*, as previously discussed. This difference in interpretation may stem in part from confusion over the type locality ("Pinehurst, Idaho"), since Pinehurst has been used for several communities within Idaho. Cronquist took this to be the Pinehurst in Blaine County, which is outside the confirmed range of *R. gymnocarpa* and also outside the range of *R. acicularis* as accepted by Lewis et al. (2015). The collector's (i.e., J. F. Macbride's) field notes in RM, however, indicate that the Pinehurst in question was instead the one in northeastern Boise County, currently marked only by an historic church on the Dry Buck Road. This area represents the southern boundary of potential *R. gymnocarpa* subsp. *helleri* habitat, though only *R. woodsii* subsp. *ultramontana* has been located at the site by the senior author during recent attempts to collect a topotype.

Although only the collection year (1912) is given in the protologue of *Rosa oligocarpa,* the cited holotype (*Eastwood 945* [A]) bears the date of 11 July. This date differs from that (29 June) on the NY sheet of *Eastwood 945*, while the corresponding CAS sheet has both dates. The type status of both sheets is accordingly in question, as is the status of those sheets (GH, POM), which give only the year.

Rosa ×collaris Rydb. (pro sp.) [= *Rosa gymnocarpa* Nutt. × *Rosa woodsii* Lindl.]**,** Flora of the Rocky Mountains 441, 1062. 1917.—TYPE: USA, Idaho [Boise Co.], Pinehurst [S of High Valley], among the streambank willows, 17 Aug 1911, *Macbride 1676* (holotype: NY; isotypes: DS, MO, NY, RM, UC).

Rosa oligocarpa Rydb., North America Flora 22: 532. 1918. —TYPE: USA, California, Shasta Co., Goose Valley, [11 Jul] 1912, *Eastwood 945* (holotype: A 32598; isotype: CAS [see discussion]).

Representative specimens. CANADA, BRITISH COLUMBIA. 15 km SE of Grand Forks, *Lewis et al. 15852* (MO). USA, CALIFORNIA. **Modoc Co.**: Rush Creek between upper and lower campgrounds, *Bartholomew 4930* (CAS, RSA); **Shasta Co.**: Goose Valley, *Eastwood 945* (TYPE of *R. oligocarpa*: A [see discussion]). USA, IDAHO. **Idaho Co.**: Shepp Ranch, Salmon River, *Davidson 11383* (SRP), Magruder Crossing on Selway River, *Christ 12782* (NY); **Latah Co.**: Troy, 28 Jun 1935, *Tucker s.n.* (CIC), valley of Little Potlatch River, *MacDougal et al. 385 p.p.* (MO); **Valley Co.**: Sylvan Beach, W side Payette Lake, *Ertter 18290* (MO, UC). MONTANA. **Flathead Co.**: Big Fork, *MacDougal 579* (NY). OREGON. **Wallowa or Union Co.**: near mouth of Minam River, *Sheldon 8664* (US).

DISPOSITION OF OTHER TYPES ASSOCIATED WITH *ROSA GYMNOCARPA*

In addition to the synonymy addressed above, other names that have been associated with *Rosa gymnocarpa* are addressed here. For the sake of completeness, all names published in Greene's 1912 paper are included, even those that otherwise have no connection to *R. gymnocarpa*. Some of this synonymy has been previously addressed by Lewis and Ertter (2007) or in Ertter's (2007) successful proposal to conserve *R. bridgesii* over several of these names. Designation of lectotypes here is perfunctory, of specimens previously accepted as holotypes but with the institution not specifically designated in the protologue.

Rosa anacantha Greene, Leaflets of Botanical Observation and Criticism 2: 265. 1912. —TYPE: USA, Washington, Pierce Co., Tacoma, 24 Aug 1889, *Greene s.n.* (holotype: NDG 11113). =*R. pisocarpa* subsp. *pisocarpa*.

Rosa breweri Greene, Leaflets of Botanical Observation and Criticism 2: 262. 1912. —TYPE: USA, California, [Santa Clara Co.], Camp 48 near San Jose, 30 Aug 1861, *Brewer 828* (lectotype, designated here: US 320924; isolectotype: POM).

Closest to *R. californica*, varying toward *R. nutkana* subsp. *nutkana*.

Rosa calvaria Greene, Leaflets of Botanical Observation and Criticism 2: 257. 1912.—TYPE: USA, California, Calaveras Co., Calaveras Big Tree grove, Jun 1889, *Greene s.n.* (holotype: NDG 023706). nom. rej. against *R. bridgesii*, =*R. bridgesii*.

Rosa copelandii Greene, Leaflets of Botanical Observation and Criticism 2: 264. 1912. —TYPE: USA, California, Siskiyou Co., Mount Eddy, 8 Sep 1903, *Copeland* [distributed as *Baker 3875*] (holotype: NDG 11152; isotypes: CAS, MO, NY, POM, RM, UC, US). =*R. pisocarpa*, intermediate between subsp. *pisocarpa* and subsp. *ahartii*. This identification differs from that of Cole (1956), who considered *R. copelandii* to be intermediate between *R. californica* and *R. pisocarpa*.

Rosa covillei Greene, Leaflets of Botanical Observation and Criticism 2: 262. 1912. —TYPE: USA, Oregon, Klamath Co., yellow pine forests south of Naylor, 22 Sep 1902, *Coville 1524* (lectotype, designated by Ertter in Taxon 56: 963. 2007: US 415341; isolectotypes: NY [fragment], US 415340). nom. rej. against *R. bridgesii*, determination ambiguous or possibly *R. bridgesii* × *R. gymnocarpa*.

Rosa crenulata Greene, Leaflets of Botanical Observation and Criticism 2: 255. 1912. —TYPE: USA, California, Fresno Co., Pine Ridge, Jun 1900, *Hall & Chandler 171* (holotype: US 390473; isotypes: K, MO, NY, UC). nom. rej. against *R. bridgesii*, =*R. bridgesii*.

Rosa delitescens Greene, Leaflets of Botanical Observation and Criticism 2: 265. 1912. —TYPE: USA, Oregon, Jackson Co., Siskiyou Mountains, 3 Sep 1889, *Greene s.n.* (holotype: NDG 23506). =*R. nutkana* subsp. *macdougalii*.

Rosa granulata Greene, Leaflets of Botanical Observation and Criticism 2: 262. 1912. —TYPE: USA, California, [San Luis Obispo Co.], San Luis Obispo, Apr 1861, *Brewer 467* (lectotype, designated here: US 320920; isolectotypes: NY [fragment], POM, UC). =*R. spithamea*, possibly with introgression from *R. californica*.

Rosa muriculata Greene, Leaflets of Botanical Observation and Criticism 2: 263. 1912. —TYPE: USA, Washington, Cowlitz Co., near Woodland, 15 Jul 1898, *Coville 705* (lectotype, effectively designated by Lewis and Ertter, Novon 17: 344. 2007: US 80003; isolectotypes: NY [fragment], US). =*R. nutkana* subsp. *nutkana*.

Rosa myriadenia Greene, Leaflets of Botanical Observation and Criticism 2: 263. 1912. —TYPE: USA, Oregon, Jackson Co., Huckleberry Mountain, 2 Aug 1897, *Coville & Applegate 368* (holotype: US 380588; isotype: NY). nom. rej. against *R. bridgesii*, determination ambiguous.

Rosa walpoleana Greene, Leaflets of Botanical Observation and Criticism 2: 264. 1912. —TYPE: USA, Oregon [Jackson Co.], Ashland, 9 Sep 1899, *F.A. Walpole s.n.* (lectotype, designated here: US 401173; isolectotype: US 401286). =*R. rubiginosa* L.

Rosa yainacensis Greene, Pittonia 5: 109. 1903. — TYPE: USA, Oregon, Klamath Co., hills of Yainax Indian Reservation, *Austin s.n.* (holotype: NDG 023696; isotype: NY). nom. rej. against *R. bridgesii*, determination ambiguous.

The type of *Rosa yainacensis* is a dwarf specimen combining features of *R. bridgesii*, *R. gymnocarpa*, and possibly other species, originating in a poorly collected part of Oregon where variation in native *Rosa* remains unresolved even after limited fieldwork by the senior author. Erlanson (1934) used the name *R. yainacensis* to encompass both *R. pinetorum* sensu Ertter (2012) and putatively tetraploid populations from Fort Ross, CA, distinct from the diploid *R. calvaria* (= *R. bridgesii*). Existing specimens annotated as *R. yainacensis* accordingly need to have identifications updated on an individual basis, including the material given this name in Bruneau et al. (2007) and Fougère-Danezan et al. (2015).

LITERATURE CITED

BOONE, L. P. 1988. Idaho place names: a geographical dictionary. University of Idaho Press, Moscow, ID.

BOULENGER, G. A. 1936. Revision des roses d'Asie sections des Eglanteriae (Suite et fin), Chinenses, Bracteatae, Banksianae et Microphyllae. Bulletin du Jardin Botanique de l'État à Bruxelles 14:115–221.

———. 1937. Étude du *Rosa gymnocarpa* Nutt. Bulletin du Jardin Botanique de l'État à Bruxelles 14:279–288.

BRUNEAU, A., J. R. STARR, AND S. JOLY. 2007. Phylogenetic relationships in the genus *Rosa*: new evidence from chloroplast DNA sequences and an appraisal of current knowledge. Systematic Botany 32:366–378.

COLE, D. 1956. A revision of the *Rosa californica* complex. American Midland Naturalist 55:211–224.

CRÉPIN, F. 1876. Primitiae Monographiae Rosarum. XII. Prodrome d'une monographie des Roses Américaines. Bulletin de la Société Royale de Botanique de Belgique 15:12–100.

———. 1889. Sketch of a new classification of roses. Journal of the Royal Horticultural Society 11:217–228.

———. 1896. Rosae Americanae I. Observations upon the genus *Rosa* in North America. Botanical Gazette 22:1–34.

CRONQUIST, A. 1961. *Rosa*. Pp. 164–171 *in* C. L. Hitchcock, A. Cronquist, M. Ownbey, and J. W. Thompson (eds.), Vascular plants of the Pacific Northwest. Part 3: Saxifragaceae to Ericaceae. University of Washington Publications in Biology 17, University of Washington Press, Seattle, WA.

——— AND N. H. HOLMGREN. 1997. *Rosa*. Pp. 134–140 *in* A. Cronquist, N. H. Holmgren, and P. K. Holmgren (eds.), Intermountain flora: vascular plants of the Intermountain West, USA. Vol. 3, Part A: Subclass Rosidae (except Fabales). New York Botanical Garden, Bronx, NY.

ERLANSON, E. W. 1930. Field observations on wild roses of the western United States. Papers of the Michigan Academy of Science, Arts & Letters 11:117–135.

———. 1934. Experimental data for a revision of the North American wild roses. Botanical Gazette 96:197–259.

ERTTER, B. 2007. Proposal to conserve the name *Rosa bridgesii* against *R. yainacensis, R. crenulata, R. calvaria, R. covillei,* and *R. myriadenia* (Rosaceae). Taxon 56:962–964.

———. 2012. *Rosa*. Pp. 1199–1202 *in* B. G. Baldwin, D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti, and D. H. Wilken (eds.), The Jepson manual: vascular plants of California, 2nd ed. University of California Press, Berkeley, CA.

——— AND W. H. LEWIS. 2008. New *Rosa* (Rosaceae) in California and Oregon. Madroño 55:170–177.

FOUGÈRE-DANEZAN, M., S. JOLY, A. BRUNEAU, X.-F. GAO, AND L.-B. ZHANG. 2015. Phylogeny and biogeography of wild roses with specific attention to polyploids. Annals of Botany 115:275–291.

GREENE, E. L. 1912. Certain western roses. Leaflets of Botanical Observation and Criticism 2:254–266.

HEATH, P. V. 1992. Preliminary notes on British roses. Calyx 1:141–195. 1992.

HENRY, J. K. 1915. Flora of southern British Columbia and Vancouver Island. W. J. Gage & Co., Toronto, Canada.

LEWIS, W. H. AND B. ERTTER. 2007. Subspecies of *Rosa nutkana* and *R. woodsii* (Rosaceae) in western North America. Novon 17:341–353.

——— AND ———. 2010. *Rosa woodsii* subsp. *puberulenta* and variety *ertterae* (Rosaceae), new in western North America. Novon 20:47–52.

———, ———, AND A. BRUNEAU. 2015 [2014]. *Rosa*. Pp. 75–119 *in* Flora of North America Editorial Committee (eds.), Flora of North America north of Mexico, Vol. 9: Magnoliophyta: Picramniaceae to Rosaceae. Oxford University Press, New York, NY.

MCKELVEY, S. D. 1955. Botanical exploration of the Trans-Mississippi West 1790–1850. Arnold Arboretum, Jamaica Plain, MA.

PIPER, C. V. 1906. Flora of the state of Washington. Contributions from the United States National Herbarium 11:1–637.

RYDBERG, P. A. 1917. Notes on Rosaceae—XI. Roses of California and Nevada. Bulletin of the Torrey Botanical Club 44:65–84.

———. 1918. *Rosa*. North American Flora 22:483–533.

PYROPIA SMITHII IS PYROPIA PULCHRA COMB. NOV.

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Some species originally placed in *Porphyra* C. Agardh were excluded from the major revision of the genus because of lack of sequenced material (Sutherland et al. 2011). Among these was *Porphyra pulchra* Hollenberg. *Porphyra pulchra* was described in Smith and Hollenberg (1943) on the basis of *Hollenberg 2890* (UC 2036529), the type specimen housed in the Herbarium at the University of California at Berkeley. This specimen (Fig. 1) consists of a blade epiphytic on *Phyllospadix* Hook that was cast ashore at Moss Beach, Pacific Grove, CA. Another specimen, UC 536729 from Santa Cruz, CA, was also referred to this species by Smith and Hollenberg (1943).

Porphyra pulchra was distinguished by its epiphytic habit, its delicately mottled violet color, and the presence of two chloroplasts in each cell. At the time of its description, this latter feature was known only in *P. lanceolata* (Setchell & Hus) G.M. Smith and *P. pulchra* among Pacific North American species in the genus. Subsequent studies have shown that the two-chloroplast condition in *P. lanceolata* is due to chloroplast division prior to cell division in the formation of reproductive cells; vegetative cells have only a single chloroplast (Lindstrom and Cole 1992). Species with two chloroplasts per vegetative cell have a much broader area separating the two chloroplasts than species in which the two-chloroplast condition precedes reproductive cell formation (e.g., compare Figs. 10 and 12 in Smith and Hollenberg 1943). After the publication of *P. pulchra*, two additional species with two chloroplasts per cell were described from the west coast of North America: *Porphyra smithii* Hollenberg & I.A. Abbott (1968, pp. 1241–1243) and *Porphyra kanakaensis* Mumford (1973, p. 239). These species were transferred to *Pyropia* as *Pyropia smithii* (G.J. Hollenberg & I.A. Abbott) S.C. Lindstrom and *P. kanakaensis* (T.F. Mumford) S.C. Lindstrom, respectively, and are not closely related (Sutherland et al. 2011).

Although it is also epiphytic [on *Mastocarpus agardhii* (Setchell & N.L. Gardner) S.C. Lindstrom, Hughey and Martone and other mid-intertidal algae], *Pyropia smithii* has not been observed on surfgrass. In contrast to *P. pulchra*, which was recorded only from the Monterey Bay and Bodega Bay areas by Abbott and Hollenberg (1976), *P. smithii* is widely distributed on the Pacific coast of North America, from the Monterey Peninsula in California to Haida Gwaii in British Columbia, Canada (Hollenberg and Abbott 1968; Kucera and Saunders 2012). It has also been collected on Calvert Island, on the central coast of British Columbia, in the low intertidal on algae-encrusted rock (UBC A90511; GenBank KT988905) as well as epiphytically on mid-intertidal *Mastocarpus* (UBC A90806; GenBank KT988908) and *Fucus* (UBC A90446, GenBank KT988906; UBC A90447, GenBank KT988904). Other differences between the species noted by Hollenberg and Abbott (1968), including tidal elevation, thallus shape, thickness and color, can be highly variable in species of foliose Bangiales.

Recently, the complete plastid genome of *Porphyra pulchra* was sequenced from a herbarium specimen collected at the type locality (UC1879714) (Lee et al. submitted; GenBank KT266789 unpublished). Dr. Hwan Su Yoon (pers. comm.) shared the *rbc*L gene sequence with us. Comparison of the *rbc*L gene of *P. pulchra* with that of *Py. smithii* shows that the sequences differ by only 2 bp. Both polymorphisms are transitions in the third codon position, accounting for less than 0.1% difference across the gene. This difference is not sufficient to warrant recognition of separate species. To confirm that the holotype specimen of *P. pulchra* is the same species as UC1879714 and *P. smithii*, we sequenced a 123 bp and a 241 bp region of the *rbc*L gene following previously described methods and precautionary guidelines (Hughey and Gabrielson 2012; Lindstrom et al. 2015). The holotype sequence (GenBank KT988909) was identical to the topotype sequence and that of *P. smithii* for these short, species-specific variable targets. *Porphyra pulchra* is the older specific epithet; we therefore make a new combination in *Pyropia* and place *P. smithii* in synonymy.

Taxonomic Treatment

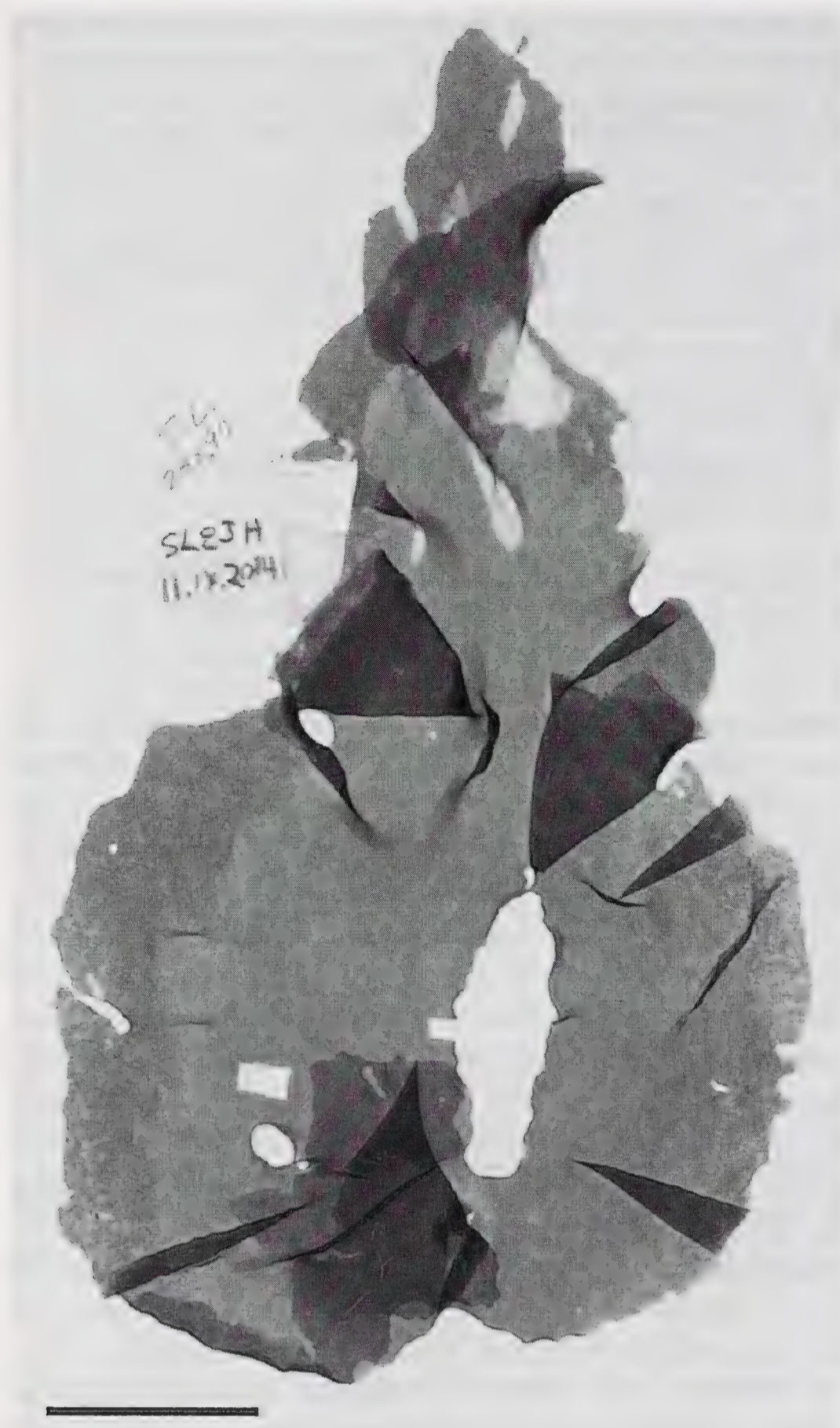***Pyropia pulchra*** (Hollenberg) S.C. Lindstrom & Hughey, sp. nov.

FIG. 1. Holotype of *Porphyra pulchra*. *Hollenberg 2980*
(UC2036529), cast ashore, epiphytic on *Phyllospadix*, Moss
Beach, Monterey Peninsula, California. Scale bar = 2 cm.

Basionym: *Porphyra pulchra* Hollenberg in Smith
and Hollenberg 1943: 213–215.

Holotype: *Hollenberg 2980* in UC 2036529.

Heterotypic synonyms: *Porphyra smithii* Hollen-
berg & I.A. Abbott 1968: 1241–1243, *Pyropia smithii*
(Hollenberg & I.A. Abbott) S.C. Lindstrom in

Sutherland et al. 2011: 1145. Holotype: US 077804,
collected by G. J. Hollenberg, Mission Point,
Monterey County, CA, USA.

LITERATURE CITED

ABBOTT, I. A. AND G. J. HOLLENBERG. 1976. Marine algae
    of California. Stanford University Press, Stanford, CA.
HOLLENBERG, G. J. AND I. A. ABBOTT. 1968. New species
    of marine algae from California. Canadian Journal of
    Botany 46:1235–1251.
HUGHEY, J. R. AND P. W. GABRIELSON. 2012. Acquiring
    DNA sequence data from dried archival red algae
    (Florideophyceae) for the purpose of applying avail-
    able names to contemporary genetic species: a critical
    assessment. Botany 90:1191–1194.
KUCERA, H. AND G. W. SAUNDERS. 2012. A survey of
    Bangiales (Rhodophyta) based on multiple molecular
    markers reveals cryptic diversity. Journal of Phycology
    48:869–882.
LINDSTROM, S. C. AND K. M. COLE. 1992. The *Porphyra
    lanceolata-P. pseudolanceolata* (Bangiales, Rhodophy-
    ta) complex unmasked: recognition of new species
    based on isozymes, morphology, chromosomes and
    distributions. Phycologia 31:431–448.
————, J. R. HUGHEY, AND L. E. AGUILAR-ROSAS. 2015.
    Four new species of *Pyropia* (Bangiales, Rhodophyta)
    from the west coast of North America: the *Pyropia
    lanceolata* species complex updated. PhytoKeys 52:1–
    22. DOI: 10.3897/phytokeys.@.5009.
MUMFORD, T. F., JR. 1973. A new species of *Porphyra*
    from the west coast of North America. Syesis 6:239–
    242.
SMITH, G. M. AND G. J. HOLLENBERG. 1943. On some
    Rhodophyceae from the Monterey Peninsula, Califor-
    nia. American Journal of Botany 30:211–222.
SUTHERLAND, J. E., S. C. LINDSTROM, W. A. NELSON, J.
    BRODIE, M. D. J. LYNCH, M. S. HWANG, H.-G. CHOI,
    M. MIYATA, N. KIKUCHI, M. C. OLIVEIRA, T. FARR,
    C. NEEFUS, A. MOLS-MORTENSEN, D. MILSTEIN,
    AND K. M. MÜLLER. 2011. A new look at ancient
    order: generic revision of the Bangiales (Rhodophyta).
    Journal of Phycology 47:1131–1151.

TWO NEW SUBSPECIES OF *ARCTOSTAPHYLOS* (ERICACEAE) FROM CALIFORNIA AND IMPLICATIONS FOR UNDERSTANDING DIVERSIFICATION IN THIS GENUS

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ABSTRACT

We describe two new subspecies of the widespread shrub *Arctostaphylos*. One subspecies is a southern extension of *A. purissima*; *A. purissima* subsp. *globosa* V.T. Parker and M.C. Vasey. It differs from the nominate species in several ways, but principally in having glandular hairs on the young stems and inflorescences and having a globose fruit typically with fused nutlets. We also describe a new subspecies of *A. patula*; *A. patula* subsp. *gankinii* M.C. Vasey and V.T. Parker. *Arctostaphylos patula* subsp. *gankinii* is a distinctive variant of the montane greenleaf manzanita that occurs widely at various localities in the Sierra Nevada extending into the inner North Coast, Klamath, and Siskiyou ranges of California and southern Oregon. This new subspecies appears to have been identified incorrectly as *A. manzanita* subsp. *roofii* and subspecies of *A. mewukka* in herbarium collections. The patterns of diversity for both species and their subspecies illustrate common taxonomic issues in the genus, representing potential inter-population hybrid introgression and intra-population morphological diversification, a potentially common mode of speciation in this genus.

Key Words: Endemism, fog influence, hybrid speciation, subspecies.

Most taxa in *Arctostaphylos* occupy a wide diversity of habitats that differ largely or subtly by climate or soil characteristics distributed in many different geographic settings. Diversification in *Arctostaphylos* appears to be driven by a number of processes involving fire regimes, soils, and climates (Vasey and Parker 2014). Additionally, climate fluctuations (Raven and Axelrod 1978), evolution of the obligate seeder life history (Wells 1969; Keeley and Zedler 1978; Parker and Kelley 1989; Parker 2015), and hybridization between species are factors likely to have played significant roles (Parker and Vasey 2004; Keeley et al. 2007; Parker et al. 2007; Vasey and Parker 2014). Frequently, parsing these different modes of diversification is difficult, although new molecular techniques may provide better insight.

In this paper we recognize two additional *Arctostaphylos* subspecies from California, one limited in geographic range to coastal western Santa Barbara County, and the other more widespread in montane habitats in the central and northern Sierra Nevada as well as inner coast range mountains of northern California and southern Oregon.

Concentrations of diversity of *Arctostaphylos* taxa between the two regions from which we describe these taxa differ considerably. The Sierra Nevada contains several wide-ranging species and few endemics, perhaps reflecting less soil diversity and a more continental climate with winter extremes. In contrast, the California outer coast ranges host the largest number of taxa in *Arctostaphylos* (Cody 1986; Keeley 1992; Vasey and Parker 2014) including numerous local endemics, probably reflecting greater edaphic diversity and a strong environmental gradient generated by a summer fog-moderated climate near the coast rapidly changing to a more interior continental climate in relatively short distances (Richerson and Lum 1980; O'Brien 1998; Francis and Currie 2003). This climatic gradient contributes to more favorable water relations for *Arctostaphylos* shrubs along the coast in contrast to more interior sites, permitting physiological diversification along a moisture water potential gradient (Vasey et al. 2012, 2014). The climatic distinctions between these two regions appear to underlie the origin of the subspecies we describe below.

THE GLOBOSE LA PURISIMA MANZANITA

This taxon was brought to our attention by several botanical consultants who were concerned that they could not confidently identify it. The first was from a site adjacent to Hwy 246 between Lompoc and Buellton, whereas the next two were from Hollister Ranch on the south slopes of the Santa Ynez Mountains to the west of Hwy 101, all from Santa Barbara County. A search of the California Consortium of Herbaria (2007) and visits to the Santa Barbara (SBBG) and Rancho Santa Ana botanic

TABLE 1. Key trait differences among *A. purissima* subspecies and two taxa to the east along the Santa Ynez Mountains.

	A. purissima subsp. *purissima*	*A. purissima* subsp. *globosa*	*A. refugioensis*	*A. glauca*
Twig pubescence	densely non-glandular short-hairy, non-glandular long-hairy	densely non-glandular short-hairy, glandular long-hairy	densely non-glandular short-hairy, glandular long-hairy	glabrous (rarely short hairs, or sparsely glandular)
Leaf length (cm)	1–2.5	2–3	3–4.5	2.5–5
Leaf width (cm)	1–2.5	1–2.5	2–3	2–4
Leaf color	green	green	glaucous-green	white glaucous
Inflorescence	raceme	raceme	5–10 branched panicle	4–8 branched panicle
Bracts/Rachis	non-glandular short-hairy (sparsely with long hair)	glandular short-hairy (sparsely with long glandular hair)	glandular short-hairy (sparsely with long glandular hair)	glabrous
Fruit diameter (mm)	4–8	4–8	10–15	10–15
Fruit shape	depressed globose	globose	globose	globose
Nutlets	separable, rugose exterior	frequently fused, rugose exterior	fused, smooth exterior	fused, smooth exterior

garden (RSA) herbaria yielded other collections that were south of Lompoc. All of these samples were glandular-hairy on their twigs and inflorescences, a condition that differs from the non-glandular nominate species located in Vandenberg Air Force Base, Burton Mesa, La Purisima Mission, and sites slightly farther north. Collections south of the Burton Mesa and Vandenberg Air Force Base differ in characteristics from the nominate species. For example, a series of collections along Jalama Road, south and west of Lompoc, all demonstrate glandular hairs on young stems and inflorescences (e.g., RSA635895, RSA635910, RSA635915, RSA635916, RSA635939) as well as a site 3.9 km west of Hwy 101 (Vasey 1468, Vasey and Parker 1489). Recently, we were able to collect this taxon in fruit from a large population at the Albolado Ranch in Santa Barbara County (34°30.727N, 120°16.052W) and discovered that, not only are stems and inflorescences covered with glandular hairs, but fruits are principally globose as well. Collections of fruit from the new subspecies in both May and July 2015 indicated that the typical fruit is globose, about 7 mm in diameter, with about half to over two-thirds of the fruit having rugose nutlets fused together. Typical *A. purissima* subsp. *purissima* have non-glandular branchlets with depressed-globose fruits and separable stones (e.g., UC1361241) (Wells 1968).

TAXONOMIC TREATMENT

Arctostaphylos purissima subsp. **globosa** V.T. Parker & M.C. Vasey, subsp. nov.—Type: USA, California, Santa Barbara Co., western Santa Ynez Mountains, mostly Matilija sandstone outcrops; 34°30'43.62"N, 120°16'03.96"W, 379 m, 24 May 2015, Parker & Vasey 1495 (holotype UCB; isotypes CAS, SFSU).

Differs from nominate species in having glandular hairs on stems and in inflorescence, fruit being globose rather than depressed globose, and frequently having fused nutlets.

Erect to spreading shrub, 2–7 m high; *stems* with burl absent; bark red-brown, smooth; *branchlets* covered in short, dense, non-glandular hairs with long, usually gland-tipped hairs; *leaves* green, isofacial, stomata equally dense on both surfaces, leaf surfaces sparsely short pubescent to glabrous with short glandular hairs along the edges, midrib, and along the petiole; blade ca. 2–3 cm long, 1.4–2.2 cm wide, oblong in shape, tips abruptly acute, base auriculate and clasping the stem; petioles < 2 mm long; *immature inflorescence* raceme supporting buds subtended by leaf-like bracts 4–5 mm long; *bracts* usually densely ciliate with some gland-tipped hairs; *flowers* conical-urceolate, whitish-pink supported by pubescent pedicels; *ovary* glabrate; *fruit* usually globose, reddish-tan, 4–8 mm wide, nutlets fused, weakly or partially fused, or sometimes separable.

Distribution and ecology. Maritime chaparral, associates include *A. glandulosa* subsp. *glandulosa*, *Pickeringia montana*, *Vaccinium ovatum*, *and Quercus agrifolia*; individuals are often adjacent to or intermixed with coastal sage scrub or oak woodland.

Etymology. The specific epithet refers to the distinct shape of the fruit compared to the nominate species, *A. purissima* subspecies *purissima*.

Taxonomic relationships. This glandular taxon suggests a zone of genetic introgressive hybrid exchange across the western portion of the Santa Ynez Mountains (Table 1). A sequence grade of taxa appears to occur from non-glandular *A. purissima* subsp. *purissima* to the north and west, then south to glandular *A. purissima* subsp. *globosa*, then to the glandular endemic *A. refugioensis* to the east, and finally to the widespread *A. glauca* farther east along the Santa Ynez Mountains and elsewhere. The typically glabrous *A. glauca* individuals in the Santa Ynez Mtns may sometimes present sparse pubes-

cence. The mosaic of characters in Table 1 suggests a few hypotheses. One is that *A. refugioensis* is of hybrid origin from a cross between an auriculate-leaved species with the large, globose-fruited *A. glauca* characterized by fused stones (Gankin 1967). The newly described subspecies of *A. purissima* with the fused globose fruit could represent introgression between *A. purissima* subsp. *purissima* and *A. refugioensis* along the northern edge of the range of the latter species.

Alternatively, historically, *A. purissima* and *A. glauca* may have dispersed into one another's populations during climatic fluctuations, even within the last 10,000 years, yielding a sequence of populations better adapted to shifts in microclimate or soils. This alternative would suggest that *A. purissima* subsp. *globosa* is of hybrid origin that

sorted back toward the auriculate parent with introgression, while *A. refugioensis* is from a similar cross that sorted back toward *A. glauca* but retained auriculate leaves and other traits. An examination of the pubescence patterns support this latter hypothesis (Figs. 1, 2). Both *A. purissima* subspecies display long stiff hairs over a matrix of generally dense shorter hair; the difference is that the longer hairs in subsp. *globosa* are glandular-tipped with small clear-to-orange or amber glands (Fig. 1). The pattern of pubescence appears to be identical between *A. purissima* subsp. *globosa* and *A. refugioensis* (Fig. 2). In addition to the character sequence, the distribution of these four taxa range from foggy maritime to sunny hot and dry interior chaparral in a similar gradient fashion, suggesting the taxa may represent an ecological adaptive sequence.

KEY TO *ARCTOSTAPHYLOS* SPECIES OF WESTERN SANTA BARBARA COUNTY AND NEARBY AREAS

1. Plants with burl at base of main stems, resprouting after fire
    2. Leaves with stomata only on lower leaf surface . . . . *A. crustacea* subsp. *eastwoodiana*
    2. Leaves with stomata on both surfaces
        3. Main stems with gray, rough, and shreddy bark . . . . *A. rudis*[1]
        3. Main stems with smooth reddish bark . . . . *A. glandulosa*
1. Plants lacking burl at base of main stems, not resprouting after fire
    4. Main stems with gray, rough, and shreddy bark . . . . *A. rudis*[2]
    4. Main stems with smooth reddish bark
        5. Leaves lobed, rounded, or slightly cuneate at base, leaves glaucous white. . . . . *A. glauca*
        5. Leaves with an auriculate base, leaf color various shades of green or gray-green
            6. Stems, immature inflorescence lacking glandular hairs
                7. Leaf blade 2–5 cm L; fruit 8–12 mm wide; stones generally fused . . . . *A. pechoensis*
                7. Leaf blade 1–2.5 cm L; fruit 5–8 mm wide; stones free . . . . *A. purissima* subsp. *purissima*
            6. Stems, immature inflorescence with glandular hairs present
                8. Leaf blade 3–4.5 cm L; leaves glaucous or dull; fruit 12–15 mm wide. . . . . *A. refugioensis*
                8. Leaf blade 1–2.5 cm L; leaves green; fruit 5–8 mm wide . . . . *A. purissima* subsp. *globosa*

THE GANKIN MANZANITA

The greenleaf manzanita (*Arctostaphylos patula* Greene) is one of the most widespread species in the genus. It is a common understory shrub in open montane yellow pine forest, white fir forest, and red fir forest and is a canopy dominant in montane chaparral, ranging along the Pacific Coast from the Cascade range of southern Washington through Oregon and the California North Coast range as far as Mt. Hull in Lake County and on both slopes of the Sierra Nevada Range, including western Nevada, then disjunct to the San Gabriel, San Bernardino, and San Jacinto Mountains of southern California, and again disjunct farther south into the Sierra San Pedro Martir in northern Baja California, Mexico. It also ranges east with a few reported occurrences in Montana and then into the Rocky Mountains from the Wasatch range, Utah through the Umcompahgre Plateau of Colorado south to the canyon lands of southern Utah and northern Arizona, and west into uplands of the Basin and Range Province of eastern Nevada.

Throughout most of this extensive range, *A. patula* presents shiny green glabrous leaves and twigs and inflorescence axes covered with dense hairs capped by distinctive stipitate golden glands (e.g., NDG37621, NDG37622, NDG37623) (Greene 1891). Whereas other species of manzanitas have glandular hairs, none have the same distinctive pattern as *A. patula*. Consequently, although its growth form varies greatly from low mounding to spreading erect shrubs, and post-fire resprouting varies geographically, the presence of these dense golden glands on twigs and inflorescence axes is generally a reliable key character to distinguish this species wherever it occurs.

However, in the upper-elevation mountain ranges of the Sierra Nevada of California, particularly in the central and northern regions, and in the inner North Coast, Siskiyou, and Klamath ranges of California and southern Oregon, individuals of *A. patula* can be found that lack these distinctive golden glands. These otherwise typical greenleaf manzanitas that lack golden glands instead present a non-glandular, short canescent tomentum on twigs and inflorescence axes. Non-glandular, canescent individuals of *A. patula*
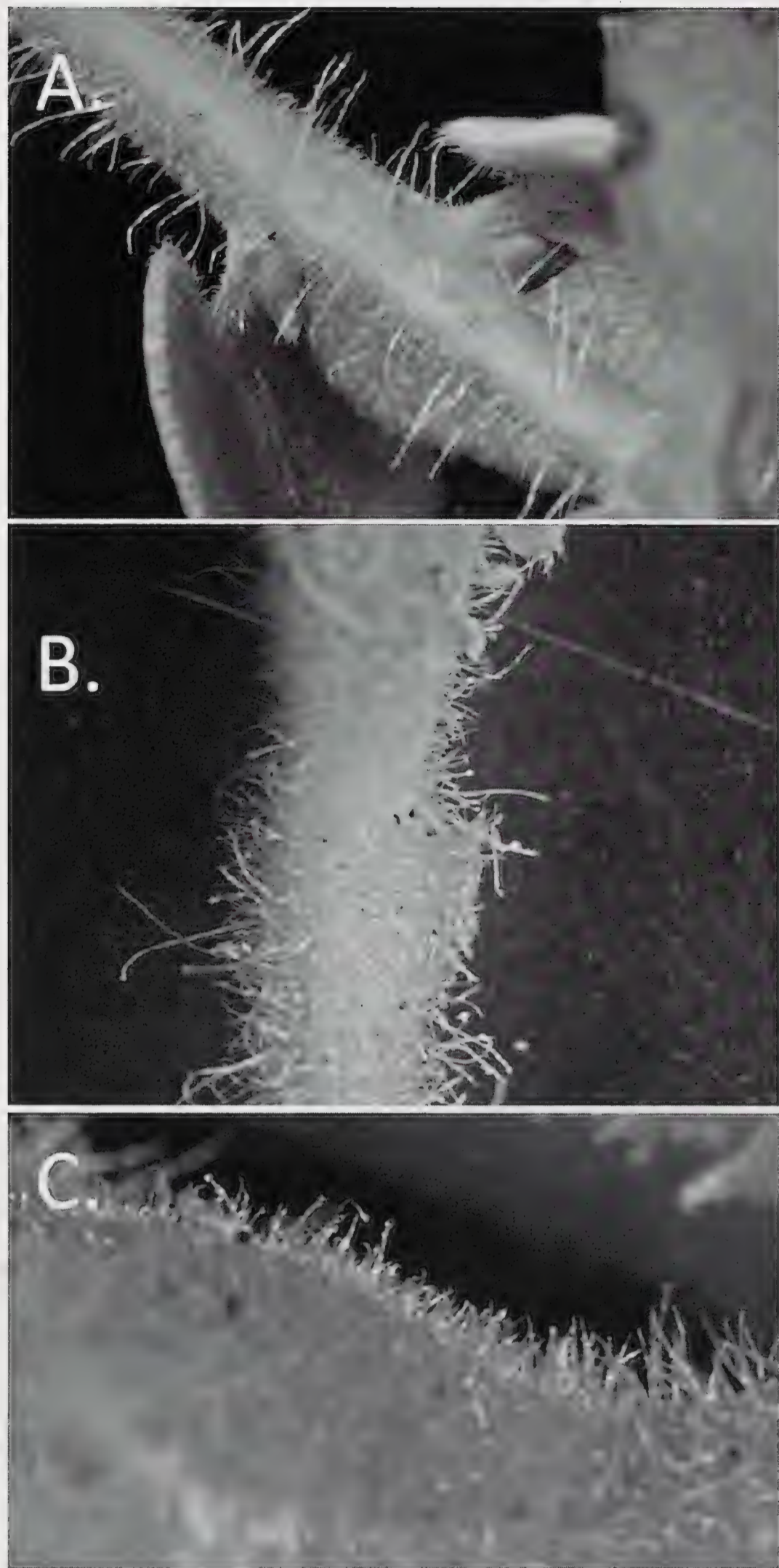
FIG. 1. *Arctostaphylos purissima* subspecies showing pubescence differences. A) stem of subsp. *purissima* showing the multi-layered pubescence; note the lack of glands at the tips of the longer hairs; B) stem hairs on *A. purissima* subsp. *globosa* illustrating the small orange glands at the tips of the longer hairs, and sometimes on the shorter hairs; C) small glands on the tips of hairs along the bracts of *A. purissima* subsp. *globosa*.
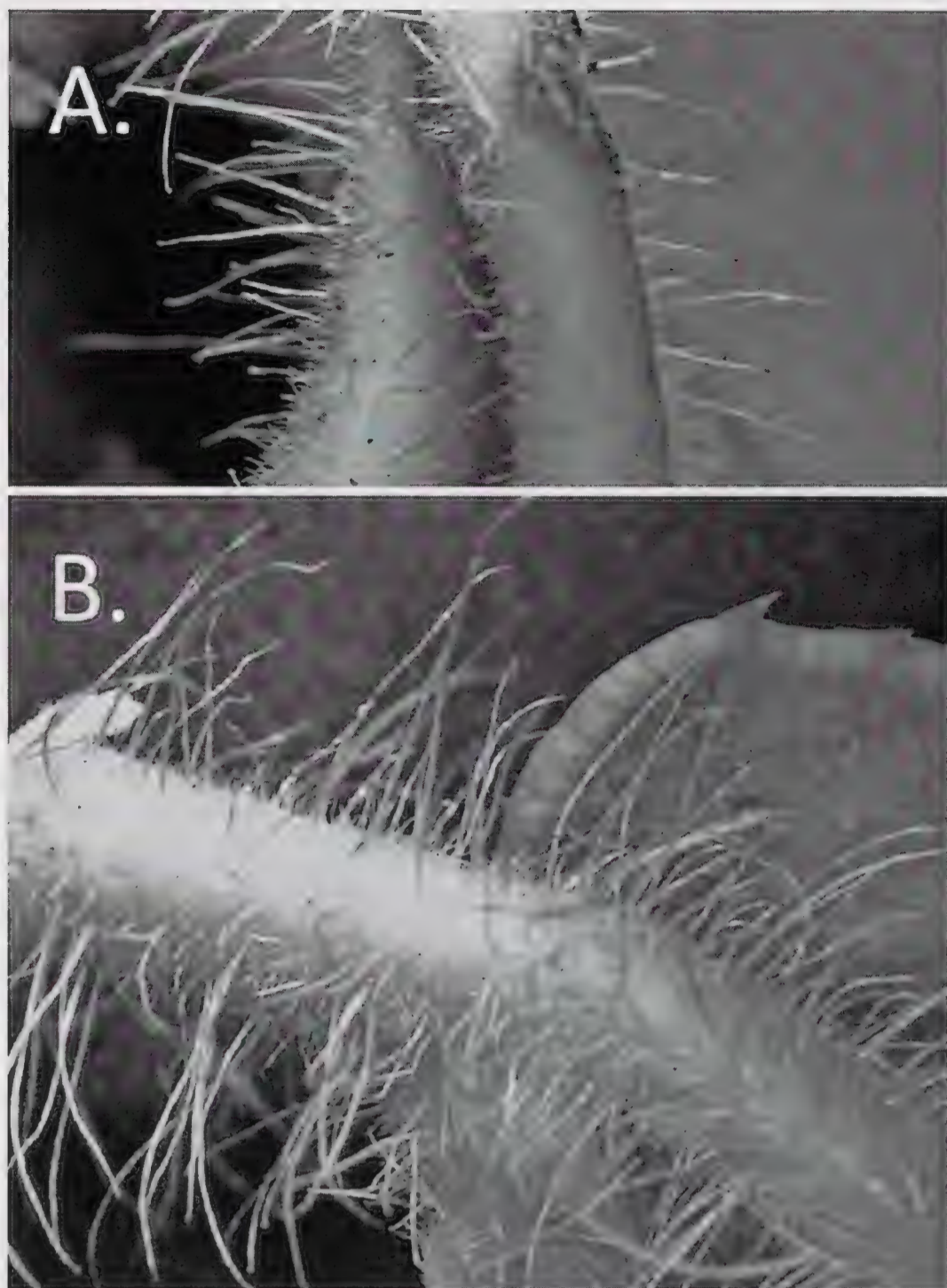

FIG. 2. Pubescence on *Arctostaphylos refugioensis*. A) pubescence at the stem-rachis transition, note the small orange glands at the tips of the longer hairs; B) pubescence along the stem with the bi-layer of hairs, glandular on the longer stiff hairs.

often occur in stands inter-mixed with typical *A. patula* and, consequently, collectors have often overlooked non-glandular *A. patula*. Accessions of non-glandular *A. patula* date back at least to a collection by W. R. Dudley in 1897 (CAS18246). Examination of herbarium specimens of *A. patula* at CAS and UC revealed approximately fifty collections of canescent, non-glandular *A. patula* by well-known botanists such as Jepson (UC38822), Eastwood (CAS202040), Abrams (CAS27374), McMinn

(UC1281165), Heller (CAS38928), Raven (CAS410293), Jepson (UC38822), Adams (UC549428), True (CAS911822), J.T. Howell (CAS863126), and others throughout the twentieth century. Although some botanists noted the undescribed canescent, non-glandular 'form', it was Roman Gankin in a 1988 collection from Highway 49 near Bassetts Station, Sierra County, CA, (CAS976495) who wrote an extensive note calling for formal recognition of this entity. Gankin, an experienced manzanita expert, mentioned that we should keep an eye out for greenleaf manzanitas that lack golden glands. We had also previously observed the canescent, non-glandular *A. patula* in 1993 along Old Highway 50 below Echo Summit, El Dorado County and along Bailey Road in Calaveras County in 1995 (e.g., Vasey 0245; Vasey 0770).

We subsequently made several visits to various stands of montane chaparral in the vicinity of the Sierra Buttes and the Gold Lakes region of Sierra and Plumas Counties and encountered large numbers of the non-glandular greenleaf manzanita, often in mixed populations with the typical glandular greenleaf manzanita. In this region, the non-glandular individuals can dominate these stands, as was demonstrated in a random survey of montane
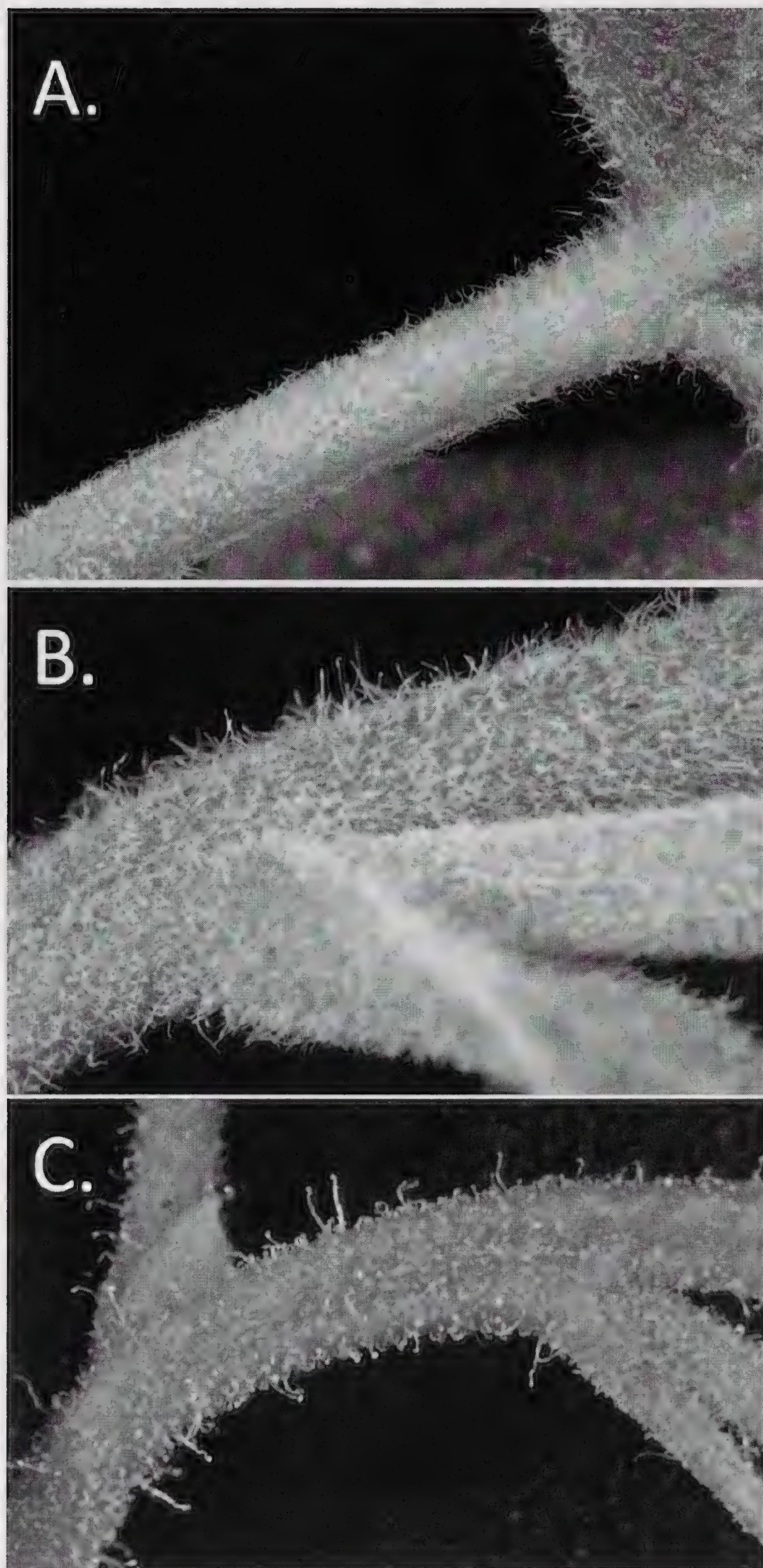
FIG. 3. Differences of stem and rachis pubescence of *Arctostaphylos patula* subspecies. A) *A. patula* subsp. *gankinii* illustrating short canescent hair on leaves, petiole and stem; B) close-up of rachis and bracts on *A. patula* subsp. *gankinii*; C) *A. patula* subsp. *patula* rachis and bracts showing the short golden glandular hairs characteristic of the species.

chaparral stands along Tamarack Trail above Upper Sardine Lake near the type locality (39°37′10″N, 120°37′35″W; 1900 m). In eight random samples, the non-glandular morph ranged from 22% to 78% of the stand. Overall, 58% of all 397 individuals sampled in this population were non-glandular.

As pointed out in the 1988 note by Gankin, the recognition of non-glandular, canescent *A. patula* mixed with typical *A. patula* was actually recorded earlier by Adams (1940) and McMinn (1939). Adams

remarked "there occurs infrequently with the typical form in the Sierra Nevada a form in which the glandular puberulence of inflorescence axes and peduncles is replaced by a light, simple pubescence. These are in complete agreement with the typical form in all other respects, including the presence of an enlarged root-crown." (Adams 1940, p. 26). Adams noted several specimens from the UC Herbarium with this character from "Modoc Co., Lassen Co., Glenn Co., Eldorado Co., Tuolumne Co., Mono Co., and Ormsby Co., Nevada." (Adams 1940, p. 26). Ormsby Co. is no longer existent and is situated within Carson City, NV. McMinn (1939) stated that the two forms "are often found associated in the same colonies (Alpine Highway from Jackson to Silver Lake)." (McMinn 1939, p. 401) (El Dorado, Co.: UC1281165, UC1281167).

Clearly, large numbers of non-glandular *A. patula* are widespread in pure or mixed populations with glandular *A. patula* over a substantial range of the Sierra Nevada, particularly the central and northern regions, as well as the inner North Coast, Siskiyou, and Klamath ranges. In at least some places, such as in the vicinity of the Sierra Buttes, non-glandular *A. patula* individuals dominate stands. This situation has created some understandable confusion with other *Arctostaphylos* taxa that have similar morphology but occur in different ecological settings, namely *A. manzanita* subsp. *roofii* (Gankin) Wells and *A. mewukka* Merriam. *Arctostaphylos manzanita* subsp. *roofii* and *A. mewukka* are also burl forming, scale-bracted, paniculate manzanitas. In *A. manzanita* subsp. *roofii*, twigs and inflorescence axes are also covered in short, canescent hairs. In *A. mewukka*, twigs and inflorescences are typically glabrous and leaves are more glaucous.

Arctostaphylos manzanita subsp. *roofii* is typically found in the inner North Coast range of Glenn and Mendocino cos.; however, it also has a disjunct occurrence in the Sierra Nevada foothills along Cohasset Road at about 500 m elevation in Butte Co. Most *A. manzanita* subsp. *roofii* collections are generally found at lower elevations (400–800 m) in chaparral and foothill woodland. However, several herbarium collections (e.g., Butte Co.: CHSC23443, UCD52219; Calaveras Co.: UCD52220; Plumas Co.:UCD52221) have been attributed to *A. manzanita* subsp. *roofii* from localities at much higher elevations (1400–1800 m) with more typical montane associates (e.g. *A. patula*, *A. nevadensis*, *Pinus ponderosa* P. Lawson and C. Lawson, *Abies magnifica* A. Murray bis, and *Calocedrus decurrens* [Torr.] Florin). Most *A. mewukka* populations occur at higher elevations than *A. manzanita* subsp. *roofii* (~1000 m); however, they typically are found below *A. patula* subsp. *patula* and *A. patula* subsp. *gankinii*. We have found collections labeled as *A. mewukka* that better match this newly described subspecies of *A. patula* in elevation, associates, and locality (e.g., *A. mewukka*, Plumas Co.: UC1408974, and *A. mewukka*, Sierra Co.: SJSU12015). Examination of

these specimens of *A. manzanita* subsp. *roofii* and *A. mewukka*, as well as their presence in high montane habitats with associates typical of *A. patula*, indicates that they most likely represent the proposed *A. patula* subsp. *gankinii*. Thus, part of our motivation in naming this subspecies is to help to provide relief from this taxonomic confusion.

TAXONOMIC TREATMENT

Arctostaphylos patula subsp. **gankinii** M.C. Vasey & V.T. Parker, subsp. nov.—Type: USA, CA, Sierra Co., south facing slope above Upper Sardine Lake near east end of lake, Tahoe National Forest, 1760 m, Sierra Nevada Mountains, 39°37′12.77″N, 120°37′02.22″W, 3 September 2006, M. Vasey1132 (holotype: UCB; isotypes: CAS, SFSU).

Differs from nominate species by having stems and inflorescence with densely short-canescent, non-glandular pubescence.

Erect to spreading shrub, 1–3 m high, *stems* with burl present, prominent to obscure; bark red-brown, smooth; *branchlets* covered in short, dense, non-glandular hairs; *leaves* erect, shiny green hue, isofacial, stomata equally dense on both surfaces; blade ca. 2.5–6 cm long, 1.5–4 cm wide, widely ovate to round, flat margins, tip abruptly soft-pointed, base rounded or truncate; petioles 0.5–1 cm long; *immature inflorescence* 2–8 branched panicle with pendent branches,

axis 1.5–3 cm; *bracts* scale-like, deltate acuminate; *flowers* conical-urceolate, whitish or pink, pedicels glabrous; *ovary* glabrous or white, non-glandular hairy; *fruit* usually depressed-globose, reddish-tan, 7–10 mm wide, glabrous, nutlets typically separable.

Paratypes. USA, CA, Calaveras Co., Dorrington, 26 June 1978, J. T. Howell (CAS911722); El Dorado Co., Baily Road, 7 October 1995, Vasey 0770; El Dorado Co., Highway 50 below Echo Summit, 13 June 1993, Vasey 0245; Plumas Co., mouth of Butterfly Creek, 10 July 1967, J.T. Howell (CAS863255); Sierra Co., montane chaparral along Highway 49 near Bassetts, 25 August 1988; R. Gankin (CAS976495).

Distribution and ecology. Occurs in mixed stands with subsp. *patula* in montane chaparral and open conifer forest between 1400–2000 m. Widespread and occasionally common in the central and northern Sierra Nevada, less common in the southern Sierra, and also found in the North Coast, Klamath, and Siskiyou ranges extending into southern Oregon. Associates include *A. nevadensis, Quercus vaccinifolia, Garrya fremontii, Ceanothus cordulatus, Prunus emarginata, Sorbus californicus, Spiraea densiflora, Pinus ponderosa, Pinus jeffreyi, Abies magnifica, and Abies concolor.*

Etymology. This subspecies is named in honor of Roman Gankin acknowledging his keen eye for manzanitas and his many contributions to understanding *Arctostaphylos* systematics.

KEY TO RESPROUTING *ARCTOSTAPHYLOS* OF MID-TO-HIGH ELEVATION MOUNTAINS IN THE WESTERN UNITED STATES AND NORTHERN BAJA CALIFORNIA

1. Leaves dull or shiny, light green to bright green in hue
    2. Plants typically prostrate, spreading or low mounding, immature inflorescence typically a short raceme (northern California to southern Oregon) . . . . *A. nevadensis* subsp. *knightii*
    2. Plants mounding to erect, immature inflorescence a wide-spreading panicle
        3. Fruits depressed-globose, stones separable or rarely fully fused
            4. Immature inflorescence bracts scale-like or awl-shaped
                5. Branchlets and immature inflorescence with dense, stipitate golden glands (mountains and high desert forests and woodland usually above snow line in western USA and northern Baja) . . . . *A. patula* subsp. *patula*
                5. Branchlets and immature inflorescence canescent, lacking stipitate golden glands
                    6. Bracts awl-shaped, high elevation sites, generally above winter snowline (Sierra Nevada, North Coast, Klamath, Siskiyou mountains, California and southern Oregon) . . . . *A. patula* subsp. *gankinii*
                    6. Bracts scale-like with marcescent tips, middle elevation sites, generally below winter snowline (inner central North Coast and Sierra mountains). . . . *A. manzanita* subsp. *roofii*
            4. Immature inflorescence bracts typically leaf-like, at least in part, especially towards the base of the rachis
                8. Stones of fruit separable or only partially fused
                    9. Branchlets and immature inflorescence densely glandular (widespread, coast range from southern Oregon to northern Baja) . . . . *A glandulosa* subsp. *glandulosa*
                    9. Branchlets and immature inflorescence canescent or with long, non-glandular hairs
                        10. Branchlets and immature inflorescence canescent, lacking long non-glandular hairs (widespread, coast range in southern Oregon to northern Baja) . . . . *A. glandulosa* subsp. *cushingiana*
                        10. Branchlets and immature inflorescence both canescent and w long, non-glandular hairs (Transverse Range, California) . . . . *A. glandulosa* subsp. *mollis*
                8. Stones of fruit fully fused (Western Transverse Range) . . . . . *A. glandulosa* subsp. *gabrielensis*

3. Fruits globose, stones typically fused
    11. Panicle multiple-branched, wide-spreading, branches and immature inflorescence sparsely glandular (southern California, Peninsular Range) . . . . *A. rainbowensis*
    11. Panicle few branched, compact, branches and immature inflorescence canescent, not glandular (Eastern Transverse Range) . . . . *A. parryana* subsp. *tumescens*
1. Leaves typically glaucous-white or gray in hue
    12. Branchlets and immature inflorescence densely glandular hairy (southern California and northern Baja) . . . .*A. glandulosa* subsp. *leucophylla*
    12. Branchlets and immature inflorescence typically non-glandular, hairy or glabrous
        13. Branchlets and immature inflorescences short hairy or canescent
            14. Fruit depressed-globose with separable stones (southern California and northern Baja) . . . . *A. glandulosa* subsp. *adamsii*
            14. Fruit typically globose with fused stones (southern California to northern Baja) . . . . *A. parryana* subsp. *desertica*
        13. Branchlets and immature inflorescence glabrous
            15. Fruit depressed-globose with separate stones
                16. Fruit 6–10 mm wide, reddish (northern Baja) . . . . *A. incognita*
                16. Fruit 10–16 mm wide, dark chocolate brown (Sierra Nevada California) . . . . *A. mewukka* subsp. *mewukka*
            15. Fruit typically with fused stones (northern Baja) . . . . *A. peninsularis* subsp. *peninsularis*

DISCUSSION

We recognize two new Californian subspecies in *Arctostaphylos*: *A. purissima* subsp. *globosa*, a rare, narrow endemic of maritime chaparral, mostly on Matilija sandstone outcrops in western Santa Barbara County in the Santa Ynez Mountains; and *A. patula* subsp. *gankinii*, an overlooked widespread and distinctive variant of the greenleaf manzanita, *A. patula*, that co-occurs with typical *A. patula* in the high mountains of the Sierra Nevada and inner coastal mountains of the North Coast, Klamath, and Siskiyou ranges of California and southern Oregon. This latter taxon is frequently confused with other taxa with similar morphology and, whereas we consider this taxon worthy of recognition on its own merits, we hope that its recognition as a subspecies will help eliminate this problem.

These two new subspecies each reflect a pattern of morphological variation found in other infra-specific taxa recognized within *Arctostaphylos*. In both cases, a distinct pattern of tomentum differs between the two taxa. In *A. purissima* subsp. *globosa*, individuals within populations are consistently glandular pubescent and also display globose fruits with typically fused stones, both in contrast to *A. purissima*. *Arctostaphylos patula* subsp. *gankinii* also exhibits a substantially different pubescent pattern from the much more broadly distributed *A. patula* subsp. *patula*, but with no other obvious morphological differences over a widespread distribution constrained to high mountains in northern California and southern Oregon.

For *A. patula* subsp. *gankinii*, the subspecies has a distinct geographic pattern that is nested within the more broadly distributed nominate species. This situation is not unique within *Arctostaphylos* and this entity historically might have been named as a variety; however, the distinction between infra-specific treatment of subspecies and varieties is now

regarded as largely unimportant by most botanists (Hamilton and Reichard 1992) and editors of floras stress a consistent treatment of one or the other infra-specific rank versus mixing the two ranks within the same genus. Other intra-population variants within *Arctostaphylos* are recognized as subspecies; e.g., the glandular *A. glandulosa* subsp. *glandulosa* versus canescent *A. glandulosa* subsp. *cushingiana* and non-glandular *A. canescens* subsp. *canescens* versus glandular *A. canescens* subsp. *sonomensis*. As we are in a time of change regarding species concepts (e.g., Soltis and Soltis 2009; Hausdorf 2011; Soltis 2013), we feel it is important to clarify the existence of this taxon. Should we choose to ignore the distinctive non-glandular variant of *A. patula*, however, its lack of recognition in floras will most likely continue the unfortunate current taxonomic confusion within the botanical community, as illustrated by *A. patula* individuals lacking golden glands that likely have been misidentified in several herbaria.

The origin of *A. patula* subsp. *gankinii* is not clear. This widespread canescent, non-glandular morph of *A. patula* has a pubescence pattern similar to populations of other manzanitas, such as *A. canescens* and *A. nissenana*, that do not co-occur with *A. patula*. The geographic movements of these other montane species since the last glacial epoch or before are not well understood and the origins of subspecies *gankinii* lie somewhere in a past we cannot yet tease apart.

Arctostaphylos purissima subsp. *globosa*, an unrecognized geographically bounded local infra-specific endemic of maritime chaparral, in contrast, morphologically suggests several possible origins. The grade of characters between *A. purissima* subsp. *purissima* to *A. glauca* (Table 1) may well represent a case of introgression between the more widespread interior *A. glauca* and *A. purissima*, with the possibility that *A. refugioensis* may also be a parent

in the mix with *A. purissima*. The putative role of homoploid hybrid origin for a taxon in a region lying between the warm, dry interior and cool, foggy coast is likely a relatively common phenomenon and other species seem to fit a similar geographic, climatic pattern, e.g., *A. gabilanensis* (Parker and Vasey 2004), and hybridization is a generally common process stimulating speciation in plants (Rieseberg 1995).

In a genus as diverse as *Arctostaphylos*, multiple pathways are likely in the origin of both species and infra-specific taxa. Bringing taxonomic coherence to the treatment of this genus requires recognizing significant entities and later research will hopefully test for different modes of origin.

Special Status Consideration

Arctostaphylos patula subsp. *gankinii* is a widespread and relatively common taxon, most frequently found on National Forest Service lands. This taxon does not seem to require special status. *Arctostaphylos purissima* subsp. *globosa*, on the other hand, is principally found on private property. Most of the land is under traditional ranching and as such is not directly threatening the chaparral stands with subsp. *globosa*. On the other hand, on the south-facing slopes of the Santa Ynez Mtns are sites associated with development, such as the former Hollister Ranch. Due to the limited geographic extent of this taxon and its patchy distribution, we consider this subspecies in need of special status consideration for CNPS Category 1b.

Literature Cited

Adams, J. E. 1940. A systematic study of the genus *Arctostaphylos* Adans. Journal of the Elisha Mitchell Scientific Society 56:1–62.

Cody, M. L. 1986. Diversity, rarity and conservation in Mediterranean-climate regions. pp. 122–152 in M.E. Soulé (ed.), Conservation biology: the science of scarcity and diversity. Sinauer Association, Sunderland, MA.

Consortium Of California Herbaria (CCH). Data provided by the participants of the Consortium of California Herbaria. Regents of the University of California 2015. Website http://ucjeps.berkeley.edu/consortium/ (14 April 2007).

Francis, A. P. and D. J. Currie. 2003. A globally consistent richness-climate relationship for angiosperms. American Naturalist 161:523–536.

Gankin, R. 1967. A new species of *Arctostaphylos* from Santa Barbara County, California. The Four Seasons 2(2):13–14.

Greene, E. L. 1891. *Arctostaphylos patula*. Pittonia 2(10):171.

Hamilton, C. W. and S. H. Reichard. 1992. Current practice in the use of subspecies, variety, and forma in the classification of wild plants. Taxon 41:485–498.

Hausdorf, B. 2011. Progress toward a general species concept. Evolution 65:923–932.

Keeley, J. E. 1992. A Californian's view of fynbos. pp. 372–378 in R. M. Cowling (ed.), The ecology of fynbos: nutrients, fire, and diversity. Oxford University Press, Capetown, South Africa.

———— and P. H. Zedler. 1978. Reproduction of chaparral shrubs after fire: a comparison of sprouting and seeding strategies. American Midland Naturalist 99:142–161.

————, M. C. Vasey, and V. T. Parker. 2007. Subspecific variation in the widespread burl-forming *Arctostaphylos glandulosa*. Madroño 54:42–62.

McMinn, H. E. 1939. An illustrated manual of California shrubs. University of California Press, Berkeley.

O'Brien, E. M. 1998. Water-energy dynamics, climate, and prediction of woody plant species richness: an interim general model. Journal of Biogeography 25:379–398.

Parker, V. T. 2015. Dispersal mutualism incorporated into large-scale, infrequent disturbances. PLoS ONE 10(7): e0132625. doi: 10.1371/journal.pone.0132625

———— and M. C. Vasey. 2004. *Arctostaphylos gabilanensis*, a newly described auriculate-leaved manzanita from the Gabilan Mountains, California. Madroño 51:322–325.

———— and V. R. Kelly. 1989. Seed banks in California chaparral and other Mediterranean climate shrublands. Pp. 231–255 in M.A. Leck, V.T. Parker, and R.L. Simpson (eds.), Ecology of soil seed banks. Academic Press, New York, NY.

————, M. C. Vasey, and J. E. Keeley. 2007. Taxonomic revisions in the genus *Arctostaphylos* (Ericaceae). Madroño 54:148–155.

Raven, P. H. and D. I. Axelrod. 1978. Origin and relationships of the California flora. University of California Publications in Botany 72:1–134.

Richerson, P. J. and K. L. Lum. 1980. Patterns of plant species diversity in California: relation to weather and topography. American Naturalist 116:504–536.

Rieseberg, L. H. 1995. The role of hybridization in evolution: old wine in new skins. American Journal of Botany 82:953–944.

Soltis, P. S. 2013. Hybridization, speciation and novelty. Journal of Evolutionary Biology 26:291–293.

———— and D. E. Soltis. 2009. The role of hybridization in plant speciation. Annual Review of Plant Biology 60:561–588.

Stebbins, G. L. and J. Major. 1965. Endemism and speciation in the California flora. Ecological Monographs 35:1–35.

Vasey, M. C. and V. T Parker. 2014. Drivers of diversity in woody plant lineages experiencing canopy fire regimes in Mediterranean type climates. Pp. 179–200 in N. Rajakaruna, R. S. Boyd, and T. B. Harris (eds.), Plant ecology and evolution in harsh environments. Nova Publishers.

————, M. E. Loik, and V. T. Parker. 2012. Influence of summer marine fog and low cloud stratus on water relations of evergreen woody shrubs (*Arctostaphylos*, Ericaceae) in the chaparral of central California. Oecologia 170:325–337.

———, V. T. PARKER, K. D. HOLL, M. E. LOIK, AND S. HIATT. 2014. Maritime climate influence on chaparral composition and diversity in the coast range of Central California. Ecology and Evolution 4(18):3662–3674.

WELLS, P. V. 1968. New taxa, combinations and chromosome numbers in Arctostaphylos (Ericaceae). Madroño 19:193–210.

———. 1969. The relation between mode of reproduction and extent of speciation in woody genera of the California chaparral. Evolution 23:264–267.

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